# POLYCHLORINATED DIBENZODIOXINS (PCDDs) AND POLYCHLORINATED DIBENZOFURANS (PCDFs) BY HIGH-RESOLUTION GAS CHROMATOGRAPHY/HIGH-RESOLUTION MASS SPECTROMETRY (HRGC/HRMS)

## 1.0 SCOPE AND APPLICATION

1.1 This method provides procedures for the detection and quantitative measurement of polychlorinated dibenzo-*p*-dioxins (tetra- through octachlorinated homologues; PCDDs), and polychlorinated dibenzofurans (tetra- through octachlorinated homologues; PCDFs) in a variety of environmental matrices and at part-per-trillion (ppt) to part-per-quadrillion (ppq) concentrations. The following compounds can be determined by this method:

| Analyte | CAS Registry No. |
|---|---|
| 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) | 1746-01-6 |
| 1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin (PeCDD) | 40321-76-4 |
| 1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin (HxCDD) | 39227-28-6 |
| 1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin (HxCDD) | 57653-85-7 |
| 1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin (HxCDD) | 19408-74-3 |
| 1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin (HpCDD) | 35822-46-9 |
| 1,2,3,4,5,6,7,8-Octachlorodibenzo-*p*-dioxin (OCDD) | 3268-87-9 |
| 2,3,7,8-Tetrachlorodibenzofuran (TCDF) | 51207-31-9 |
| 1,2,3,7,8-Pentachlorodibenzofuran (PeCDF) | 57117-41-6 |
| 2,3,4,7,8-Pentachlorodibenzofuran (PeCDF) | 57117-31-4 |
| 1,2,3,4,7,8-Hexachlorodibenzofuran (HxCDF) | 70648-26-9 |
| 1,2,3,6,7,8-Hexachlorodibenzofuran (HxCDF) | 57117-44-9 |
| 1,2,3,7,8,9-Hexachlorodibenzofuran (HxCDF) | 72918-21-9 |
| 2,3,4,6,7,8-Hexachlorodibenzofuran (HxCDF) | 60851-34-5 |
| 1,2,3,4,6,7,8-Heptachlorodibenzofuran (HpCDF) | 67562-39-4 |
| 1,2,3,4,7,8,9-Heptachlorodibenzofuran (HpCDF) | 55673-89-7 |
| 1,2,3,4,5,6,7,8-Octachlorodibenzofuran (OCDF) | 39001-02-0 |
| Total Tetrachlorodibenzo-*p*-dioxin (TCDD) | 41903-57-5 |
| Total Pentachlorodibenzo-*p*-dioxin (PeCDD) | 36088-22-9 |
| Total Hexachlorodibenzo-*p*-dioxin (HxCDD) | 34465-46-8 |
| Total Heptachlorodibenzo-*p*-dioxin (HpCDD) | 37871-00-4 |
| Total Tetrachlorodibenzofuran (TCDF) | 55722-27-5 |
| Total Pentachlorodibenzofuran (PeCDF) | 30402-15-4 |
| Total Hexachlorodibenzofuran (HxCDF) | 55684-94-1 |
| Total Heptachlorodibenzofuran (HpCDF) | 38998-75-3 |

1.2 The analytical method calls for the use of high-resolution gas chromatography and high-resolution mass spectrometry (HRGC/HRMS) on purified sample extracts. Table 1 lists the various sample types covered by this analytical protocol, the 2,3,7,8-TCDD-based method calibration limits (MCLs), and other pertinent information. Samples containing concentrations of specific congeneric analytes (PCDDs and PCDFs) considered within the scope of this method that are greater than ten times the upper MCLs must be analyzed by a protocol designed for such concentration levels, e.g., Method 8280. An optional method for reporting the analytical results using a 2,3,7,8-TCDD toxicity equivalency factor (TEF) is described.

1.3 The sensitivity of this method is dependent upon the level of interferences within a given matrix. The calibration range of the method for a 1-L water sample is 10 to 2000 ppq for TCDD/TCDF and PeCDD/PeCDF, and 1.0 to 200 ppt for a 10-g soil, sediment, fly ash, or tissue sample for the same analytes (Table 1). Analysis of a one-tenth aliquot of the sample permits measurement of concentrations up to 10 times the upper MCL. The actual limits of detection and quantitation will differ from the lower MCL, depending on the complexity of the matrix.

1.4 This method is designed for use by analysts who are experienced with residue analysis and skilled in HRGC/HRMS.

1.5 Because of the extreme toxicity of many of these compounds, the analyst must take the necessary precautions to prevent exposure to materials known or believed to contain PCDDs or PCDFs. It is the responsibility of the laboratory personnel to ensure that safe handling procedures are employed. Sec. 11 of this method discusses safety procedures.

## 2.0 SUMMARY OF METHOD

2.1 This procedure uses matrix-specific extraction, analyte-specific cleanup, and HRGC/HRMS analysis techniques.

2.2 If interferences are encountered, the method provides selected cleanup procedures to aid the analyst in their elimination. A simplified analysis flow chart is presented at the end of this method.

2.3 A specified amount (see Table 1) of soil, sediment, fly ash, water, sludge (including paper pulp), still bottom, fuel oil, chemical reactor residue, fish tissue, or human adipose tissue is spiked with a solution containing specified amounts of each of the nine isotopically ($^{13}C_{12}$) labeled PCDDs/PCDFs listed in Column 1 of Table 2. The sample is then extracted according to a matrix-specific extraction procedure. Aqueous samples that are judged to contain 1 percent or more solids, and solid samples that show an aqueous phase, are filtered, the solid phase (including the filter) and the aqueous phase extracted separately, and the extracts combined before extract cleanup. The extraction procedures are:

a) Toluene: Soxhlet extraction for soil, sediment, fly ash, and paper pulp samples;
b) Methylene chloride: liquid-liquid extraction for water samples;
c) Toluene: Dean-Stark extraction for fuel oil, and aqueous sludge samples;
d) Toluene extraction for still bottom samples;
e) Hexane/methylene chloride: Soxhlet extraction or methylene chloride: Soxhlet extraction for fish tissue samples; and
f) Methylene chloride extraction for human adipose tissue samples.

g) As an option, all solid samples (wet or dry) may be extracted with toluene using a Soxhlet/Dean Stark extraction system or using pressurized fluid extraction (PFE) (Method 3545).

The decision for the selection of an extraction procedure for chemical reactor residue samples is based on the appearance (consistency, viscosity) of the samples. Generally, they can be handled according to the procedure used for still bottom (or chemical sludge) samples.

2.4 The extracts are submitted to an acid-base washing treatment and dried. Following a solvent exchange step, the extracts are cleaned up by column chromatography on alumina, silica gel, and activated carbon.

2.4.1 The extracts from adipose tissue samples are treated with silica gel impregnated with sulfuric acid before chromatography on acidic silica gel, neutral alumina, and activated carbon.

2.4.2 Fish tissue and paper pulp extracts are subjected to an acid wash treatment only, prior to chromatography on alumina and activated carbon.

2.5 The preparation of the final extract for HRGC/HRMS analysis is accomplished by adding 10 to 50 µL (depending on the matrix) of a nonane solution containing 50 pg/µL of the recovery standards $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD (Table 2). The former is used to determine the percent recoveries of tetra- and pentachlorinated PCDD/PCDF congeners, while the latter is used to determine the percent recoveries of the hexa-, hepta- and octachlorinated PCDD/PCDF congeners.

2.6 A 2-µL aliquot of the concentrated extract are injected into an HRGC/HRMS system capable of performing selected ion monitoring at resolving power of at least 10,000 (10 percent valley definition).

2.7 The identification of OCDD and nine of the fifteen 2,3,7,8-substituted congeners (Table 3), for which $^{13}C$-labeled standards are available in the sample fortification and recovery standard solutions (Table 2), is based on their elution at their exact retention time (within 0.005 retention time units measured in the routine calibration) and the simultaneous detection of the two most abundant ions in the molecular ion region. The remaining six 2,3,7,8-substituted congeners (i.e., 2,3,4,7,8-PeCDF; 1,2,3,4,7,8-HxCDD; 1,2,3,6,7,8-HxCDF; 1,2,3,7,8,9-HxCDF; 2,3,4,6,7,8-HxCDF, and 1,2,3,4,7,8,9-HpCDF), for which no carbon-labeled internal standards are available in the sample fortification solution, and all other PCDD/PCDF congeners are identified when their relative retention times fall within their respective PCDD/PCDF retention time windows, as established from the routine calibration data, and the simultaneous detection of the two most abundant ions in the molecular ion region. The identification of OCDF is based on its retention time relative to $^{13}C_{12}$-OCDD and the simultaneous detection of the two most abundant ions in the molecular ion region. Identification also is based on a comparison of the ratios of the integrated ion abundance of the molecular ion species to their theoretical abundance ratios.

2.8 Quantitation of the individual congeners, total PCDDs, and total PCDFs is achieved in conjunction with the establishment of a multipoint (five points) calibration curve for each homologue, during which each calibration solution is analyzed once.

## 3.0 INTERFERENCES

3.1 Solvents, reagents, glassware and other sample processing hardware may yield discrete artifacts or elevated baselines that may cause misinterpretation of the chromatographic data (see references 1 and 2.) All of these materials must be demonstrated to be free from interferants under the conditions of analysis by performing laboratory method blanks. Analysts should avoid using PVC gloves.

3.2 The use of high purity reagents and solvents helps minimize interference problems. Purification of solvents by distillation in all-glass systems may be necessary.

3.3 Interferants coextracted from the sample will vary considerably from matrix to matrix. PCDDs and PCDFs are often associated with other interfering chlorinated substances such as polychlorinated biphenyls (PCBs), polychlorinated diphenyl ethers (PCDPEs), polychlorinated naphthalenes, and polychlorinated alkyldibenzofurans, that may be found at concentrations several orders of magnitude higher than the analytes of interest. Retention times of target analytes must be verified using reference standards. These values must correspond to the retention time windows established in Sec. 8.1.1.3. While cleanup techniques are provided as part of this method, unique samples may require additional cleanup steps to achieve lower detection limits.

3.4 A high-resolution capillary column (60-m DB-5, J&W Scientific, or equivalent) is used in this method. However, no single column is known to resolve all isomers. The 60-m DB-5 GC column is capable of 2,3,7,8-TCDD isomer specificity (Sec. 8.1.1). In order to determine the concentration of the 2,3,7,8-TCDF (if detected on the DB-5 column), the sample extract must be reanalyzed on a column capable of 2,3,7,8-TCDF isomer specificity (e.g., DB-225, SP-2330, SP-2331, or equivalent).

## 4.0 APPARATUS AND MATERIALS

4.1 High-resolution gas chromatograph/high-resolution mass spectrometer/data system (HRGC/HRMS/DS) - The GC must be equipped for temperature programming, and all required accessories must be available, such as syringes, gases, and capillary columns.

4.1.1 GC injection port - The GC injection port must be designed for capillary columns. The use of splitless injection techniques is recommended. On-column 1-µL injections can be used on the 60-m DB-5 column. The use of a moving needle injection port is also acceptable. When using the method described in this protocol, a 2-µL injection volume is used consistently (i.e., the injection volumes for all extracts, blanks, calibration solutions and the performance check samples are 2 µL). The use of 1-µL injections is allowed; however, laboratories must remain consistent throughout the analyses by using the same injection volume at all times.

4.1.2 GC/MS interface - The GC/MS interface components should withstand 350°C. The interface must be designed so that the separation of 2,3,7,8-TCDD from the other TCDD isomers achieved in the gas chromatographic column is not appreciably degraded. Cold spots or active surfaces (adsorption sites) in the GC/MS interface can cause peak tailing and peak broadening. It is recommended that the GC column be fitted directly into the mass spectrometer ion source without being exposed to the ionizing electron beam. Graphite ferrules should be avoided in the injection port because they may adsorb the PCDDs and PCDFs. Vespel™, or equivalent, ferrules are recommended.

4.1.3 Mass spectrometer - The static resolving power of the instrument must be maintained at a minimum of 10,000 (10 percent valley).

4.1.4 Data system - A dedicated data system is employed to control the rapid multiple-ion monitoring process and to acquire the data. Quantitation data (peak areas or peak heights) and SIM traces (displays of intensities of each ion signal being monitored including the lock-mass ion as a function of time) must be acquired during the analyses and stored. Quantitations may be reported based upon computer-generated peak areas or upon measured peak heights (chart recording). The data system must be capable of acquiring data at a minimum of 10 ions in a single scan. It is also recommended to have a data system capable of switching to different sets of ions (descriptors) at specified times during an HRGC/HRMS acquisition. The data system should be able to provide hard copies of individual ion chromatograms for selected gas chromatographic time intervals. It should also be able to acquire mass spectral peak profiles (Sec. 8.1.2.3) and provide hard copies of peak profiles to demonstrate the required resolving power. The data system should permit the measurement of noise on the base line.

NOTE: The detector ADC zero setting must allow peak-to-peak measurement of the noise on the base line of every monitored channel and allow for good estimation of the instrument resolving power. The effect of different zero settings on the measured resolving power is shown in Figure 2.

4.2 GC columns

Fused-silica capillary columns are needed. The columns shall demonstrate the required separation of all 2,3,7,8-specific isomers whether a dual-column or a single-column analysis is chosen. Chromatographic performance must be demonstrated and documented (Sec. 8.2.2) at the beginning of each 12-hour period (after mass resolution and GC resolution are demonstrated) during which sample extracts or concentration calibration solutions will be analyzed. Recommended operating conditions for the recommended columns are shown in Sec. 7.6.

4.2.1 60-m DB-5 (J&W Scientific) or equivalent fused-silica capillary column

In order to have an isomer-specific determination of 2,3,7,8-TCDD and to allow the detection of OCDD/OCDF within a reasonable time interval in one HRGC/HRMS analysis, use of this column is recommended. Isomer specificity for all 2,3,7,8-substituted PCDDs/PCDFs cannot be achieved on the 60-m DB-5 column. Problems have been associated with the separation of 2,3,7,8-TCDD from 1,2,3,7-TCDD and 1,2,6,8-TCDD, and separation of 2,3,7,8-TCDF from 1,2,4,9-, 1,2,7,9-, 2,3,4,6-, 2,3,4,7-, and 2,3,4,8-TCDF. Because of the toxicologic concern associated with 2,3,7,8-TCDD and 2,3,7,8-TCDF, additional analyses may be necessary for some samples.

4.2.2 30-m DB-225 (J&W Scientific) or equivalent fused-silica capillary column

For the DB-225 column, problems are associated with the separation of 2,3,7,8-TCDF from 2,3,4,7-TCDF and a combination of 1,2,3,9- and 2,3,4,8-TCDF.

4.3 Miscellaneous equipment and materials - The following list of items does not necessarily constitute an exhaustive compendium of the equipment needed for this analytical method.

NOTE: Reuse of glassware should be minimized to avoid the risk of contamination. All glassware that is reused should be scrupulously cleaned as soon as possible after use, according to the following procedure: Rinse glassware with the last solvent used in it. Wash with hot detergent water, then rinse with copious amounts of tap water and several portions of organic-free reagent water. Rinse with high purity acetone and hexane and store it inverted or capped with solvent rinsed aluminum foil in a clean environment.

4.3.1 Nitrogen evaporation apparatus with variable flow rate.

4.3.2 Balances capable of accurately weighing to 0.01 g and 0.0001 g.

4.3.3 Centrifuge.

4.3.4 Water bath - equipped with concentric ring covers and capable of being temperature controlled within ± 2°C.

4.3.5 Stainless steel or glass container large enough to hold contents of one-pint sample containers.

4.3.6 Glove box.

4.3.7 Drying oven.

4.3.8 Stainless steel spoons and spatulas.

4.3.9 Laboratory hoods.

4.3.10 Pipets - disposable, Pasteur, 150 mm long x 5 mm ID.

4.3.11 Pipets - disposable, serological, 10-mL, for the preparation of the carbon columns specified in Sec. 7.5.3.

4.3.12 Reaction vial - 2-mL, silanized amber glass (Reacti-vial, or equivalent).

4.3.13 Stainless steel meat grinder with a 3 to 5 mm hole size inner plate.

4.3.14 Separatory funnels - 125-mL and 2000-mL.

4.3.15 Kuderna-Danish concentrator - 500-mL, fitted with 10-mL concentrator tube and three-ball Snyder column.

4.3.16 PTFE or Carborundum (silicon carbide) boiling chips (or equivalent), washed with hexane before use.

NOTE: PFTE boiling chips may float in methylene chloride, may not work in the presence of any water phase, and may be penetrated by nonpolar organic compounds.

4.3.17 Chromatographic columns - glass, 300 mm x 10.5 mm, fitted with PTFE stopcock.

4.3.18 Adapters for concentrator tubes.

4.3.19 Glass fiber filters - 0.70-µm, Whatman GFF, or equivalent.

4.3.20 Dean-Stark trap - 5- or 10-mL, with T-joints, condenser and 125-mL flask.

4.3.21 Continuous liquid-liquid extractor - 1-L sample capacity, suitable for use with heavier than water solvents.

4.3.22 All glass Soxhlet apparatus with 500-mL flask.

4.3.23 Soxhlet/Dean-Stark extractor (optional) - all glass, 500-mL flask.

4.3.24 Glass funnels - sized to hold 170 mL of liquid.

4.3.25 Desiccator.

4.3.26 Solvent reservoir (125-mL) - compatible with gravity carbon column.

4.3.27 Rotary evaporator with a temperature-controlled water bath.

4.3.28 High speed tissue homogenizer - equipped with an EN-8 probe, or equivalent.

4.3.29 Glass wool - extract with methylene chloride, dry, and store in a glass jar.

4.3.30 Extraction jars - glass, 250-mL, with PTFE-lined screw cap.

4.3.31 Volumetric flasks - Class A, 10-mL to 1000-mL.

4.3.32 Glass vials - 1-dram (or metric equivalent).

## 5.0 REAGENTS AND STANDARD SOLUTIONS

5.1 Organic-free reagent water - All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

5.2 Column chromatography reagents

5.2.1 Alumina - neutral, 80/200 mesh (Super 1, Woelm®, or equivalent). Store in a sealed container at room temperature, in a desiccator, over self-indicating silica gel.

5.2.2 Alumina - acidic AG4, (Bio Rad Laboratories catalog #132-1240, or equivalent). Soxhlet extract with methylene chloride for 24 hours if blanks show contamination, and activate by heating in a foil covered glass container for 24 hours at 190°C. Store in a glass bottle sealed with a PTFE-lined screw cap.

5.2.3 Silica gel - high purity grade, type 60, 70-230 mesh. Soxhlet extract with methylene chloride for 24 hours if blanks show contamination, and activate by heating in a foil

covered glass container for 24 hours at 190°C. Store in a glass bottle sealed with a PTFE-lined screw cap.

5.2.4 Silica gel impregnated with sodium hydroxide. Add one part (by weight) of 1 M NaOH solution to two parts (by weight) silica gel (extracted and activated) in a screw cap bottle and mix with a glass rod until free of lumps. Store in a glass bottle sealed with a PTFE-lined screw cap.

5.2.5 Silica gel impregnated with 40 percent (by weight) sulfuric acid. Add two parts (by weight) concentrated sulfuric acid to three parts (by weight) silica gel (extracted and activated), mix with a glass rod until free of lumps, and store in a screw capped glass bottle. Store in a glass bottle sealed with a PTFE-lined screw cap.

5.2.6 Celite 545® (Supelco), or equivalent.

5.2.7 Charcoal carbon - Activated carbon, Carbopak C (Supelco) or equivalent, prewashed with methanol and dried *in vacuo* at 110°C. Store in a glass bottle sealed with a PTFE-lined screw cap. (Note: AX-21 [Anderson Development Company] carbon is no longer available, but existing stocks may be utilized).

5.3 Reagents

5.3.1 Sulfuric acid, $H_2SO_4$, concentrated, ACS grade, specific gravity 1.84.

5.3.2 Potassium hydroxide, KOH, ACS grade, 20 percent (w/v) in organic-free reagent water.

5.3.3 Sodium chloride, NaCl, analytical reagent, 5 percent (w/v) in organic-free reagent water.

5.3.4 Potassium carbonate, $K_2CO_3$, anhydrous, analytical reagent.

5.4 Sodium sulfate (powder, anhydrous), $Na_2SO_4$ - Purify by heating at 400°C for 4 hours in a shallow tray. If, after heating, the sodium sulfate develops a noticeable grayish cast (due to the presence of carbon in the crystal matrix) that batch of sodium sulfate is not suitable for use and should be discarded. Extraction with methylene chloride may produce sodium sulfate that is suitable for use in such instances, but following extraction, a reagent blank must be analyzed that demonstrates that there is no interference from the sodium sulfate.

5.5 Solvents - all solvents must be (at a minimum) pesticide grade or equivalent, distilled-in-glass.

5.5.1 Methylene chloride, $CH_2Cl_2$.

5.5.2 Hexane, $C_6H_{14}$.

5.5.3 Methanol, $CH_3OH$.

5.5.4 Nonane, $C_9H_{20}$.

5.5.5 Toluene, $C_6H_5CH_3$.

5.5.6 Cyclohexane, $C_6H_{12}$.

5.5.7 Acetone, $CH_3C0CH_3$.

5.6 High-Resolution Concentration Calibration Solutions (Table 5) - Five nonane solutions containing 17 unlabeled and 11 carbon-labeled PCDDs and PCDFs at known concentrations are used to calibrate the instrument. The concentration ranges are homologue-dependent, with the lowest values for the tetrachlorinated dioxin and furan (1.0 pg/µL) and the highest values for the octachlorinated congeners (1000 pg/µL). Standards containing more carbon-labeled PCDDs and PCDFs may also be employed.

5.7 GC Column Performance Check Solution - This solution contains the first and last eluting isomers for each homologous series from tetra- through heptachlorinated congeners. The solution also contains a series of other TCDD isomers for the purpose of documenting the chromatographic resolution. The $^{13}C_{12}$-2,3,7,8-TCDD is also present. The laboratory is required to use nonane as the solvent and adjust the volume so that the final concentration does not exceed 100 pg/µL per congener. Table 7 summarizes the qualitative composition (minimum requirement) of this performance evaluation solution.

5.8 Sample Fortification Solution - This nonane solution contains the nine internal standards at the nominal concentrations that are listed in Table 2. The solution contains at least one carbon-labeled standard for each homologous series, and it is used to measure the concentrations of the native substances. (Note that $^{13}C_{12}$-OCDF is not present in the solution.) Standards containing more carbon-labeled PCDDs and PCDFs may also be employed, provided that the same labeled compounds are contained in the calibration standards in Sec. 5.6.

5.9 Recovery Standard Solution - This nonane solution contains two recovery standards, $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD, at a nominal concentration of 50 pg/µL per compound. 10 to 50 µL of this solution will be spiked into each sample extract before the final concentration step and HRGC/HRMS analysis.

5.10 Matrix Spike Fortification Solution - Solution used to prepare the MS and MSD samples. It contains all unlabeled analytes listed in Table 5 at concentrations corresponding to the HRCC 3.

## 6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 See the introductory material to this chapter, Organic Analytes, Sec. 4.1.

6.2 Sample collection

6.2.1 Sample collection personnel should, to the extent possible, homogenize samples in the field before filling the sample containers. This should minimize or eliminate the necessity for sample homogenization in the laboratory. The analyst should make a judgment, based on the appearance of the sample, regarding the necessity for additional mixing. If the sample is clearly not homogeneous, the entire contents should be transferred to a glass or stainless steel pan for mixing with a stainless steel spoon or spatula before removal of a sample portion for analysis.

6.2.2 Grab and composite samples must be collected in glass containers. Conventional sampling practices must be followed. The bottle must not be prewashed with

sample before collection. Sampling equipment must be free of potential sources of contamination.

6.3 Grinding or blending of fish samples - If not otherwise specified in a project plan, the whole fish (frozen) should be blended or ground to provide a homogeneous sample. The use of a stainless steel meat grinder with a 3 to 5 mm hole size inner plate is recommended. In some circumstances, analysis of fillet or specific organs of fish may be requested. If so requested, the above whole fish requirement is superseded.

6.4 Storage and holding times - All samples, except fish and adipose tissue samples, must be stored at 4°C in the dark, and should be extracted within 30 days and completely analyzed within 45 days of extraction. Fish and adipose tissue samples must be stored at -20°C in the dark, and should be extracted within 30 days and completely analyzed within 45 days of collection.

NOTE: The holding times listed in Sec. 6.4 are recommendations. PCDDs and PCDFs are very stable in a variety of matrices, and holding times under the conditions listed in Sec. 6.4 may be as high as a year for certain matrices.

6.5 Phase separation

This is a guideline for phase separation for very wet (>25 percent water) soil, sediment and paper pulp samples. Place a 50-g portion in a suitable centrifuge bottle and centrifuge for 30 minutes at 2,000 rpm. Remove the bottle and mark the interface level on the bottle. Estimate the relative volume of each phase. With a disposable pipet, transfer the liquid layer into a clean bottle. Mix the solid with a stainless steel spatula and remove a portion to be weighed and analyzed (percent dry weight determination, extraction). Return the remaining solid portion to the original sample bottle (empty) or to a clean sample bottle that is properly labeled, and store it as appropriate. Analyze the solid phase by using only the soil, sediment and paper pulp method. Take note of, and report, the estimated volume of liquid before disposing of the liquid as a liquid waste.

6.6 Soil, sediment, or paper sludge (pulp) percent dry weight determination

When results are to be reported on a dry-weight basis, the percent dry weight of soil, sediment or paper pulp samples may be determined according to the following procedure. Weigh a 10-g portion of the soil or sediment sample (± 0.5 g) to three significant figures. Dry it to constant weight at 110°C in an adequately ventilated oven. Allow the sample to cool in a desiccator. Weigh the dried solid to three significant figures. Calculate and report the percent dry weight. Do not use this solid portion of the sample for extraction, but instead dispose of it as hazardous waste.

$$\%\ \text{dry weight} = \frac{\text{g of dry sample}}{\text{g of sample}} \times 100$$

CAUTION: Finely divided soils and sediments contaminated with PCDDs/PCDFs are hazardous because of the potential for inhalation or ingestion of particles containing PCDDs/PCDFs (including 2,3,7,8-TCDD). Such samples should be handled in a confined environment (i.e., a closed hood or a glove box).

6.7 Lipid content determination

6.7.1 Fish tissue - To determine the lipid content of fish tissue, concentrate 125 mL of the fish tissue extract (Sec. 7.2.2), in a tared 200-mL round-bottom flask, on a rotary evaporator until a constant weight (W) is achieved.

$$\% \text{ lipid} = \frac{\text{weight of residue} \times 2}{\text{weight of sample}} \times 100$$

The factor of 2 accounts for the use of half of the extract (e.g., 125 mL of 250 mL total volume) for the lipid determination.

Dispose of the lipid residue as a hazardous waste if the results of the analysis indicate the presence of PCDDs or PCDFs.

Other procedures and other extract volumes may be employed for the lipid determination, provided that they are clearly described and documented. Adjustments to the amount of internal standards spiked in Sec. 7.1 will be required if different volumes are employed.

6.7.2 Adipose tissue - Details for the determination of the adipose tissue lipid content are provided in Sec. 7.3.3.

## 7.0 PROCEDURE

7.1 Internal standard addition

The sample fortification solution (Sec. 5.8) containing the carbon-labeled internal standards is added to each sample prior to extraction.

7.1.1 Select an appropriate size sample aliquot. Typical sample size requirements for different matrices are given in Sec. 7.4 and in Table 1. Transfer the sample portion to a tared flask and determine its weight.

7.1.2 Except for adipose tissue, add an appropriate quantity of the sample fortification mixture (Sec. 5.8) to the sample. All samples should be spiked with 100 µL of the sample fortification mixture to give internal standard concentrations as indicated in Table 1. As an example, for $^{13}C_{12}$-2,3,7,8-TCDD, a 10-g soil sample requires the addition of 1000 pg of $^{13}C_{12}$-2,3,7,8-TCDD to give the required 100 ppt fortification level. The fish tissue sample (20 g) must be spiked with 200 µL of the internal standard solution, because half of the extract will be used to determine the lipid content (Sec. 6.7.1).

7.1.2.1 For the fortification of soil, sediment, fly ash, water, fish tissue, paper pulp and wet sludge samples, mix the sample fortification solution with 1.0 mL acetone.

7.1.2.2 Do not dilute the nonane solution for the other matrices.

7.1.2.3 The fortification of adipose tissue is carried out at the time of homogenization (Sec. 7.3.2.3).

7.2 Extraction and purification of fish and paper pulp samples

7.2.1 Add 60 g of anhydrous sodium sulfate to a 20-g portion of a homogeneous fish sample (Sec. 6.3) and mix thoroughly with a stainless steel spatula. After breaking up any lumps, place the fish/sodium sulfate mixture in the Soxhlet apparatus on top of a glass wool plug. Add 250 mL methylene chloride or hexane/methylene chloride (1:1) to the Soxhlet apparatus and reflux for 16 hours. The solvent must cycle completely through the system five times per hour. Follow the same procedure for the partially dewatered paper pulp sample (using a 10-g sample, 30 g of anhydrous sodium sulfate and 200 mL of toluene).

NOTE: As an option, a Soxhlet/Dean-Stark extractor system may be used, with toluene as the solvent. No sodium sulfate is added when using this option.

7.2.2 Transfer the fish extract from Sec. 7.2.1 to a 250-mL volumetric flask and fill to the mark with methylene chloride. Mix well, then remove 125 mL for the determination of the lipid content (Sec. 6.7.1). Transfer the remaining 125 mL of the extract, plus two 15-mL hexane/methylene chloride rinses of the volumetric flask, to a K-D apparatus equipped with a Snyder column. Quantitatively transfer all of the paper pulp extract to a K-D apparatus equipped with a Snyder column.

NOTE: As an option, a rotary evaporator may be used in place of the K-D apparatus for the concentration of the extracts.

7.2.3 Add a PTFE (or equivalent) boiling chip. Concentrate the extract in a water bath to an apparent volume of 10 mL. Remove the apparatus from the water bath and allow to cool for 5 minutes.

7.2.4 Add 50 mL hexane and a new boiling chip to the K-D flask. Concentrate in a water bath to an apparent volume of 5 mL. Remove the apparatus from the water bath and allow to cool for 5 minutes.

NOTE: The methylene chloride must have been completely removed before proceeding with the next step.

7.2.5 Remove and invert the Snyder column and rinse it into the K-D apparatus with two 1-mL portions of hexane. Decant the contents of the K-D apparatus and concentrator tube into a 125-mL separatory funnel. Rinse the K-D apparatus with two additional 5-mL portions of hexane and add the rinses to the funnel. Proceed with the cleanup according to the instructions in Sec. 7.5.1.1, but omit the procedures described in Secs. 7.5.1.2 and 7.5.1.3.

7.3 Extraction and purification of human adipose tissue

7.3.1 Human adipose tissue samples must be stored at a temperature of -20°C or lower from the time of collection until the time of analysis. The use of chlorinated materials during the collection of the samples must be avoided. Samples are handled with stainless steel forceps, spatulas, or scissors. All sample bottles (glass) are cleaned as specified in the note at the end of Sec. 4.3. PTFE-lined caps should be used.

NOTE: The specified storage temperature of -20°C is the maximum storage temperature permissible for adipose tissue samples. Lower storage temperatures are recommended.

7.3.2 Adipose tissue extraction

7.3.2.1 Weigh a 10-g portion of a frozen adipose tissue sample to the nearest 0.01 g, into a culture tube (2.2 x 15 cm).

NOTE: The sample size may be smaller, depending on availability. In such situations, the analyst is required to adjust the volume of the internal standard solution added to the sample to meet the fortification level stipulated in Table 1.

7.3.2.2 Allow the adipose tissue specimen to reach room temperature (up to 2 hours).

7.3.2.3 Add 10 mL of methylene chloride and 100 µL of the sample fortification solution. Homogenize the mixture for approximately 1 minute with a tissue homogenizer.

7.3.2.4 Allow the mixture to separate, then remove the methylene chloride extract from the residual solid material with a disposable pipet. Percolate the methylene chloride through a filter funnel containing a clean glass wool plug and 10 g of anhydrous sodium sulfate. Collect the dried extract in a graduated 100-mL volumetric flask.

7.3.2.5 Add a second 10 mL portion of methylene chloride to the sample and homogenize for 1 minute. Decant the solvent, dry it, and transfer it to the 100-mL volumetric flask (Sec. 7.3.2.4).

7.3.2.6 Rinse the culture tube with at least two additional portions of methylene chloride (10-mL each), and transfer the entire contents to the filter funnel containing the anhydrous sodium sulfate. Rinse the filter funnel and the anhydrous sodium sulfate contents with additional methylene chloride (20 to 40 mL) into the 100-mL flask. Discard the sodium sulfate.

7.3.2.7 Adjust the volume to the 100-mL mark with methylene chloride.

7.3.3 Adipose tissue lipid content determination

7.3.3.1 Preweigh a clean 1-dram (or metric equivalent) glass vial to the nearest 0.0001 g on an analytical balance tared to zero.

7.3.3.2 Accurately transfer 1.0 mL of the final extract (100 mL) from Sec. 7.3.2.7 to the vial. Reduce the volume of the extract on a water bath (50-60°C) by a gentle stream of purified nitrogen until an oily residue remains. Nitrogen evaporation is continued until a constant weight is achieved.

NOTE: When the sample size of the adipose tissue is smaller than 10 g, then the analyst may use a larger portion (up to 10 percent) of the extract defined in Sec. 7.3.2.7 for the lipid determination.

7.3.3.3 Accurately weigh the vial with the residue to the nearest 0.0001 g and calculate the weight of the lipid present in the vial based on the difference of the weights.

7.3.3.4 Calculate the percent lipid content of the original sample to the nearest 0.1 percent as shown below:

$$\% \text{ Lipid} = \frac{W_{lr} \times V_{ext}}{W_{at} \times V_{al}} \times 100$$

where:

$W_{lr}$ = weight of the lipid residue to the nearest 0.0001 g calculated from Sec. 7.3.3.3,

$V_{ext}$ = total volume (100 mL) of the extract in mL from Sec. 7.3.2.7,

$W_{at}$ = weight of the original adipose tissue sample to the nearest 0.01 g from Sec. 7.3.2.1, and

$V_{al}$ = volume of the aliquot of the final extract in mL used for the quantitative measure of the lipid residue (1.0 mL) from Sec. 7.3.3.2.

7.3.3.5 Record the weight of the lipid residue measured in Sec. 7.3.3.3 and the percent lipid content from Sec. 7.3.3.4.

7.3.4 Adipose tissue extract concentration

7.3.4.1 Quantitatively transfer the remaining extract from Sec. 7.3.3.2 (99.0 mL) to a 500-mL Erlenmeyer flask. Rinse the volumetric flask with 20 to 30 mL of additional methylene chloride to ensure quantitative transfer.

7.3.4.2 Concentrate the extract on a rotary evaporator and a water bath at 40°C until an oily residue remains.

7.3.5 Adipose tissue extract cleanup

7.3.5.1 Add 200 mL of hexane to the lipid residue in the 500-mL Erlenmeyer flask and swirl the flask to dissolve the residue.

7.3.5.2 Slowly add, with stirring, 100 g of 40 percent (w/w) sulfuric acid-impregnated silica gel. Stir with a magnetic stirrer for two hours at room temperature.

7.3.5.3 Allow the solid phase to settle, and decant the liquid through a filter funnel containing 10 g of anhydrous sodium sulfate on a glass wool plug, into another 500-mL Erlenmeyer flask.

7.3.5.4 Rinse the solid phase with two 50-mL portions of hexane. Stir each rinse for 15 minutes, decant, and dry as described under Sec. 7.3.5.3. Combine the hexane extracts from Sec. 7.3.5.3 with the rinses.

7.3.5.5 Rinse the sodium sulfate in the filter funnel with an additional 25 mL of hexane and combine this rinse with the hexane extracts from Sec. 7.3.5.4.

7.3.5.6 Prepare an acidic silica column as follows: Pack a 2 cm x 10 cm chromatographic column with a glass wool plug, add approximately 20 mL of hexane, add 1 g of silica gel and allow to settle, then add 4 g of 40 percent (w/w) sulfuric acid-impregnated silica gel and allow to settle. Elute the excess hexane from the column until the solvent level reaches the top of the chromatographic packing. Verify that the column does not have any air bubbles and channels.

7.3.5.7 Quantitatively transfer the hexane extract from the Erlenmeyer flask (Secs. 7.3.5.3 through 7.3.5.5) to the silica gel column reservoir. Allow the hexane extract to percolate through the column and collect the eluate in a 500-mL K-D apparatus.

7.3.5.8 Complete the elution by percolating 50 mL of hexane through the column into the K-D apparatus. Concentrate the eluate on a steam bath to about 5 mL. Use nitrogen evaporation to bring the final volume to about 100 µL.

NOTE: If the silica gel impregnated with 40 percent sulfuric acid is highly discolored throughout the length of the adsorbent bed, the cleaning procedure must be repeated beginning with Sec. 7.3.5.1.

7.3.5.9 The extract is ready for the column cleanups described in Secs. 7.5.2 through 7.5.3.6.

7.4 Extraction and purification of environmental and waste samples

7.4.1 Sludge/wet fuel oil

7.4.1.1 Extract aqueous sludge or wet fuel oil samples by refluxing a sample (e.g., 2 g) with 50 mL of toluene in a 125-mL flask fitted with a Dean-Stark water separator. Continue refluxing the sample until all the water is removed.

NOTE: If the sludge or fuel oil sample dissolves in toluene, treat it according to the instructions in Sec. 7.4.2 below. If the sludge sample originates from pulp (paper mills), treat it according to the instructions starting in Sec. 7.2, but without the addition of sodium sulfate.

7.4.1.2 Cool the sample, filter the toluene extract through a glass fiber filter, or equivalent, into a 100-mL round-bottom flask.

7.4.1.3 Rinse the filter with 10 mL of toluene and combine the extract with the rinse.

7.4.1.4 Concentrate the combined solutions to near dryness on a rotary evaporator at 50°C or using nitrogen evaporation. Proceed with Sec. 7.4.4.

7.4.2 Still bottom/oil

7.4.2.1 Extract still bottom or oil samples by mixing a sample portion (e.g., 1.0 g) with 10 mL of toluene in a small beaker and filtering the solution through a glass fiber filter (or equivalent) into a 50-mL round-bottom flask. Rinse the beaker and filter with 10 mL of toluene.

7.4.2.2 Concentrate the combined toluene solutions to near dryness on a rotary evaporator at 50°C or using nitrogen evaporation. Proceed with Sec. 7.4.4.

7.4.3 Fly ash

NOTE: Because of the tendency of fly ash to "fly", all handling steps should be performed in a hood in order to minimize contamination.

7.4.3.1 Weigh about 10 g of fly ash to two decimal places and transfer to an extraction jar. Add 100 µL of the sample fortification solution (Sec. 5.8), diluted to 1 mL with acetone, to the sample. Add 150 mL of 1 M HCl to the fly ash sample. Seal the jar with the PTFE-lined screw cap and shake for 3 hours at room temperature.

7.4.3.2 Rinse a glass fiber filter with toluene, and filter the sample through the filter paper, placed in a Buchner funnel, into a 1-L flask. Wash the fly ash cake with approximately 500 mL of organic-free reagent water and dry the filter cake overnight at room temperature in a desiccator.

7.4.3.3 Add 10 g of anhydrous powdered sodium sulfate, mix thoroughly, let sit in a closed container for one hour, mix again, let sit for another hour, and mix again.

7.4.3.4 Place the sample and the filter paper into an extraction thimble, and extract in a Soxhlet extraction apparatus charged with 200 mL of toluene for 16 hours using a five cycle/hour schedule.

NOTE: As an option, a Soxhlet/Dean-Stark extractor system may be used, with toluene as the solvent. No sodium sulfate is added when using this option.

7.4.3.5 Cool and filter the toluene extract through a glass fiber filter into a 500-mL round-bottom flask. Rinse the filter with 10 mL of toluene. Add the rinse to the extract and concentrate the combined toluene solutions to near dryness on a rotary evaporator at 50°C or using nitrogen evaporation. Proceed with Sec. 7.4.4.

7.4.3.6 Alternatively, fly ash samples may be extracted with a toluene/acetic acid mixture using pressurized fluid extraction (PFE), as described in Method 3545. When using PFE, the HCl pretreatment in Sec. 7.4.3.1 may be omitted.

7.4.4 Transfer the concentrate to a 125-mL separatory funnel using 15 mL of hexane. Rinse the flask with two 5-mL portions of hexane and add the rinses to the funnel. Shake the combined solutions in the separatory funnel for two minutes with 50 mL of 5 percent sodium chloride solution, discard the aqueous layer, and proceed with Sec. 7.5.

7.4.5 Aqueous samples

7.4.5.1 Allow the sample to come to ambient temperature, then mark the water meniscus on the side of the 1-L sample bottle for later determination of the exact sample volume. Add the required acetone diluted sample fortification solution (Sec. 5.8).

7.4.5.2 When the sample is judged to contain 1 percent or more solids, the sample must be filtered through a glass fiber filter that has been rinsed with toluene. If the suspended solids content is too great to filter through the 0.45-µm filter, centrifuge the sample, decant, and then filter the aqueous phase.

NOTE: Paper mill effluent samples normally contain 0.02%-0.2% solids, and would not require filtration. However, for optimum analytical results, all paper mill effluent samples should be filtered, the isolated solids and filtrate extracted separately, and the extracts recombined.

7.4.5.3 Combine the solids from the centrifuge bottle(s) with the particulates on the filter and with the filter itself and proceed with the Soxhlet extraction as specified in Secs. 7.4.6.1 through 7.4.6.4.

NOTE: Pressurized fluid extraction has *not* been evaluated for the extraction of the particulate fraction.

Remove and invert the Snyder column and rinse it down into the K-D apparatus with two 1-mL portions of hexane.

7.4.5.4 Pour the aqueous filtrate into a 2-L separatory funnel. Add 60 mL of methylene chloride to the sample bottle, seal and shake for 30 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting.

7.4.5.5 Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation (e.g., glass stirring rod).

7.4.5.6 Collect the methylene chloride in a K-D apparatus (mounted with a 10-mL concentrator tube) by passing the sample extracts through a filter funnel packed with a glass wool plug and 5 g of anhydrous sodium sulfate.

NOTE: As an option, a rotary evaporator may be used in place of the K-D apparatus for the concentration of the extracts.

7.4.5.7 Repeat the extraction twice with fresh 60-mL portions of methylene chloride. After the third extraction, rinse the sodium sulfate with an additional 30 mL of methylene chloride to ensure quantitative transfer. Combine all extracts and the rinse in the K-D apparatus.

NOTE: A continuous liquid-liquid extractor may be used in place of a separatory funnel when experience with a sample from a given source indicates that a serious emulsion problem will result or an emulsion is encountered when using a separatory funnel. Add 60 mL of methylene chloride to the sample bottle, seal, and shake for 30 seconds to rinse the inner surface. Transfer

the solvent to the extractor. Repeat the rinse of the sample bottle with an additional 50- to 100-mL portion of methylene chloride and add the rinse to the extractor. Add 200 to 500 mL of methylene chloride to the distilling flask, add sufficient organic-free reagent water (Sec. 5.1) to ensure proper operation, and extract for 24 hours. Allow to cool, then detach the distilling flask. Dry and concentrate the extract as described in Secs. 7.4.5.6 and 7.4.5.8 through 7.4.5.10. Proceed with Sec. 7.4.5.11.

7.4.5.8 Attach a Snyder column and concentrate the extract on a water bath until the apparent volume of the liquid is 5 mL. Remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

7.4.5.9 Remove the Snyder column, add 50 mL of hexane, add the concentrate obtained from the Soxhlet extraction of the suspended solids (Sec. 7.4.5.3), if applicable, re-attach the Snyder column, and concentrate to approximately 5 mL. Add a new boiling chip to the K-D apparatus before proceeding with the second concentration step.

7.4.5.10 Rinse the flask and the lower joint with two 5-mL portions of hexane and combine the rinses with the extract to give a final volume of about 15 mL.

7.4.5.11 Determine the original sample volume by filling the sample bottle to the mark with water and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL. Proceed with Sec. 7.5.

7.4.6 Soil/sediment

7.4.6.1 Add 10 g of anhydrous powdered sodium sulfate to the sample aliquot (10 g or less) and mix thoroughly with a stainless steel spatula. After breaking up any lumps, place the soil/sodium sulfate mixture in the Soxhlet apparatus on top of a glass wool plug (the use of an extraction thimble is optional).

NOTE: As an option, a Soxhlet/Dean-Stark extractor system may be used, with toluene as the solvent. No sodium sulfate is added when using this option.

7.4.6.2 Add 200 to 250 mL of toluene to the Soxhlet apparatus and reflux for 16 hours. The solvent must cycle completely through the system five times per hour.

NOTE: If the dried sample is not of free flowing consistency, more sodium sulfate must be added.

7.4.6.3 Cool and filter the extract through a glass fiber filter into a 500-mL round-bottom flask for evaporation of the toluene. Rinse the filter with 10 mL of toluene, and concentrate the combined fractions to near dryness on a rotary evaporator at 50°C. Remove the flask from the water bath and allow to cool for 5 minutes.

7.4.6.4 Transfer the residue to a 125-mL separatory funnel, using 15 mL of hexane. Rinse the flask with two additional portions of hexane, and add the rinses to the funnel. Proceed with Sec. 7.5.

7.4.6.5 Alternatively, soil/sediment samples may be extracted with toluene using pressurized fluid extraction (PFE), as described in Method 3545.

7.5 Cleanup

7.5.1 Acid-base washing

7.5.1.1 Partition the hexane extract against 40 mL of concentrated sulfuric acid. Shake for two minutes. Remove and discard the sulfuric acid layer (bottom). Repeat the acid washing until no color is visible in the acid layer (perform a maximum of four acid washings).

7.5.1.2 Omit this step for the fish sample extract. Partition the extract against 40 mL of 5 percent (w/v) aqueous sodium chloride. Shake for two minutes. Remove and discard the aqueous layer (bottom).

7.5.1.3 Omit this step for the fish sample extract. Partition the extract against 40 mL of 20 percent (w/v) aqueous potassium hydroxide (KOH). Shake for two minutes. Remove and discard the aqueous layer (bottom). Repeat the base washing until no color is visible in the bottom layer (perform a maximum of four base washings). Strong base (KOH) is known to degrade certain PCDDs/PCDFs, so contact time must be minimized.

7.5.1.4 Partition the extract against 40 mL of 5 percent (w/v) aqueous sodium chloride. Shake for two minutes. Remove and discard the aqueous layer (bottom). Dry the extract by pouring it through a filter funnel containing anhydrous sodium sulfate on a glass wool plug, and collect it in a 50-mL round-bottom flask. Rinse the funnel with the sodium sulfate with two 15-mL portions of hexane, add the rinses to the 50-mL flask, and concentrate the hexane solution to near dryness on a rotary evaporator (35°C water bath) or nitrogen evaporation, making sure all traces of toluene (when applicable) are removed.

7.5.2 Silica/alumina column cleanup

7.5.2.1 Pack a gravity column (glass, 30 cm x 10.5 mm), fitted with a PTFE stopcock, with of silica gel as follows: Insert a glass wool plug into the bottom of the column. Place 1 g of silica gel in the column and tap the column gently to settle the silica gel. Add 2 g of sodium hydroxide-impregnated silica gel, 4 g of sulfuric acid-impregnated silica gel, and 2 g of silica gel. Tap the column gently after each addition. A small positive pressure (5 psi) of clean nitrogen may be used if needed. Elute with 10 mL of hexane and close the stopcock just before exposure of the top layer of silica gel to air. Discard the eluate. Check the column for channeling. If channeling is observed, discard the column. Do not tap the wetted column.

7.5.2.2 Pack a gravity column (glass, 300 mm x 10.5 mm), fitted with a PTFE stopcock, with alumina as follows: Insert a glass wool plug into the bottom of the column. Add a 4 g layer of sodium sulfate. Add a 4 g layer of Woelm® Super 1 neutral alumina. Tap the top of the column gently. Woelm® Super 1 neutral alumina need not be activated or cleaned before use, but it should be stored in a sealed desiccator. Add a 4 g layer of anhydrous sodium sulfate to cover the alumina. Elute with 10 mL hexane and close the stopcock just before exposure of the sodium sulfate

layer to air. Discard the eluate. Check the column for channeling. If channeling is observed, discard the column. Do not tap a wetted column.

NOTE: Alternatively, acidic alumina (Sec. 5.2.2) may be used in place of neutral alumina.

7.5.2.3 Dissolve the residue from Sec. 7.5.1.4 in 2 mL of hexane and apply the hexane solution to the top of the silica gel column. Rinse the flask with enough hexane (3-4 mL) to quantitatively transfer of the sample to the surface of the silica gel.

7.5.2.4 Elute the silica gel column with 90 mL of hexane, concentrate the eluate on a rotary evaporator (35°C water bath) to approximately 1 mL, and apply the concentrate to the top of the alumina column (Sec. 7.5.2.2). Rinse the rotary evaporator flask twice with 2 mL of hexane, and add the rinses to the top of the alumina column.

7.5.2.5 Add 20 mL of hexane to the alumina column and elute until the hexane level is just below the top of the sodium sulfate. Do not discard the eluted hexane, but collect it in a separate flask and store it for later use, as it may be useful in determining where the labeled analytes are being lost if recoveries are not satisfactory.

7.5.2.6 Add 15 mL of 60 percent methylene chloride in hexane (v/v) to the alumina column and collect the eluate in a conical-shaped (15-mL) concentration tube. With a carefully regulated stream of nitrogen, concentrate the 60 percent methylene chloride/hexane fraction to about 2 mL.

7.5.3 Carbon column cleanup

7.5.3.1 Thoroughly mix 9.0 g of activated carbon (Sec. 5.2.7) and 41.0 g of Celite 545® to produce an 18% w/w mixture. Activate the mixture at 130°C for 6 hours, and store in a desiccator.

NOTE: Check each new batch of the carbon/Celite mixture by adding 50 µL of the calibration verification solution to 950 µL of hexane. Take this solution through the carbon column cleanup step, concentrate to 50 µL and analyze. If the recovery of any of the analytes is less than 80%, this batch of carbon/Celite mixture may not be used.

7.5.3.2 Prepare a 4-inch long glass column by cutting off each end of a 10-mL disposable serological pipet. Fire polish both ends and flare if desired. Insert a glass wool plug at one end of the column, and pack it with 1 g of the carbon/Celite mixture. Insert an additional glass wool plug in the other end.

CAUTION: It is very important that the column be packed properly to ensure that carbon fines are not carried into the eluate. PCDDs/PCDFs will adhere to the carbon fines and greatly reduce recovery. If carbon fines are carried into the eluate, filter the eluate, using a 0.7-µm filter (pre-rinsed with toluene), then proceed to Sec. 7.5.3.6.

7.5.3.3 Rinse the column with:

4 mL of toluene
2 mL of methylene chloride/methanol/toluene (75:20:5 v/v)
4 mL of cyclohexane/methylene chloride (50:50 v/v)

The flow rate should be less than 0.5 mL/min. Discard all the column rinsates.

7.5.3.4 While the column is still wet, transfer the concentrated eluate from Sec. 7.5.2.6 to the prepared carbon column. Rinse the eluate container with two 0.5-mL portions of hexane and transfer the rinses to the carbon column. Elute the column with the following sequence of solvents.

10 mL of cyclohexane/methylene chloride (50:50 v/v).
5 mL of methylene chloride/methanol/toluene (75:20:5 v/v).

NOTE: The above two eluates may be collected and combined, and used as a check on column efficiency.

7.5.3.5 Once the solvents have eluted through the column, turn the column over, and elute the PCDD/PCDF fraction with 20 mL of toluene, and collect the eluate.

7.5.3.6 Concentrate the toluene fraction to about 1 mL on a rotary evaporator by using a water bath at 50°C or with nitrogen evaporation. Carefully transfer the concentrate into a 1-mL minivial and, again at elevated temperature (50°C), reduce the volume to about 100 µL using a stream of nitrogen and a sand bath. Rinse the rotary evaporator flask three times with 300 µL of a solution of 1 percent toluene in methylene chloride, and add the rinses to the concentrate. Add 10 µL of the nonane recovery standard solution (Sec. 5.9) for soil, sediment, water, fish, paper pulp and adipose tissue samples, or 50 µL of the recovery standard solution for sludge, still bottom and fly ash samples. Store the sample at room temperature in the dark.

7.6 Chromatographic/mass spectrometric conditions and data acquisition parameters

7.6.1 Gas chromatograph operating conditions

| | |
|---|---|
| Column coating: | DB-5 |
| Film thickness: | 0.25 µm |
| Column dimension: | 60-m x 0.32 mm |
| Injector temperature: | 270°C |
| Splitless valve time: | 45 s |
| Interface temperature: | Function of the final temperature |
| | |
| Temperature program | |
| Initial temperature: | 200°C |
| Initial hold time: | 2 min |
| 1st temp. ramp: | 5 °C/min to 220°C, hold for 16 minutes |
| 2nd temp. ramp: | 5 °C/min to 235°C, hold for 7 minutes |
| 3rd temp. ramp: | 5 °C/min to 330°C, hold for 5 minutes |

Total time: 60 min

7.6.2 Mass spectrometer

7.6.2.1 The mass spectrometer must be operated in a selected ion monitoring (SIM) mode with a total cycle time (including the voltage reset time) of one second or less (Sec. 7.6.3.1). At a minimum, the ions listed in Table 6 for each of the five SIM descriptors must be monitored. Note that with the exception of the last descriptor (OCDD/OCDF), all descriptors contain 10 ions. The selection (Table 6) of the molecular ions M and M+2 for $^{13}$C-HxCDF and $^{13}$C-HpCDF rather than M+2 and M+4 (for consistency) was made to eliminate, even under high-resolution mass spectrometric conditions, interferences occurring in these two ion channels for samples containing high levels of native HxCDDs and HpCDDs. It is important to maintain the same set of ions for both calibration and sample extract analyses. The selection of the lock-mass ion is left to the performing laboratory.

NOTE: At the option of the analyst, the tetra- and pentachlorinated dioxins and furans may be combined into a single descriptor.

7.6.2.2 The recommended mass spectrometer tuning conditions are based on the groups of monitored ions shown in Table 6. By using a PFK molecular leak, tune the instrument to meet the minimum required resolving power of 10,000 (10 percent valley) at m/z 304.9824 (PFK) or any other reference signal close to m/z 303.9016 (from TCDF). By using peak matching conditions and the aforementioned PFK reference peak, verify that the exact mass of m/z 380.9760 (PFK) is within 5 ppm of the required value. Note that the selection of the low- and high-mass ions must be such that they provide the largest voltage jump performed in any of the five mass descriptors (Table 6).

7.6.3 Data acquisition

7.6.3.1 The total cycle time for data acquisition must be $\leq$ 1 second. The total cycle time includes the sum of all the dwell times and voltage reset times.

7.6.3.2 Acquire SIM data for all of the ions in the descriptors in Table 6.

7.7 Calibration

7.7.1 Initial calibration

Initial calibration is required before any samples are analyzed for PCDDs and PCDFs and must meet the acceptance criteria in Sec. 7.7.2. Initial calibration is also required if any routine calibration (Sec. 7.7.3) does not meet the required criteria listed in Sec. 7.7.2.

7.7.1.1 All five high-resolution concentration calibration solutions listed in Table 5 must be used for the initial calibration.

7.7.1.2 Tune the instrument with PFK, as described in Sec. 7.6.2.2.

7.7.1.3 Inject 2 µL of the GC column performance check solution (Sec. 5.7) and acquire SIM mass spectral data as described earlier in Sec. 7.6.2. The total cycle time must be $\leq$ 1 second. The laboratory must not perform any further analysis until it is demonstrated and documented that the criteria listed in Sec. 8.2.1 were met.

7.7.1.4 By using the same GC (Sec. 7.6.1) and MS (Sec. 7.6.2) conditions that produced acceptable results with the column performance check solution, analyze a 2-µL portion of each of the five concentration calibration solutions once with the following mass spectrometer operating parameters.

7.7.1.4.1 The ratio of integrated ion current for the ions appearing in Table 8 (homologous series quantitation ions) must be within the indicated control limits (set for each homologous series) for all unlabeled calibration standards in Table 5.

7.7.1.4.2 The ratio of integrated ion current for the ions belonging to the carbon-labeled internal and recovery standards (Table 5) must be within the control limits stipulated in Table 8.

NOTE: Secs. 7.7.1.4.1 and 7.7.1.4.2 require that 17 ion ratios from Sec. 7.7.1.4.1 and 11 ion ratios from Sec. 7.7.1.4.2 be within the specified control limits simultaneously in one run. It is the laboratory's responsibility to take corrective action if the ion abundance ratios are outside the limits.

7.7.1.4.3 For each selected ion current profile (SICP) and for each GC signal corresponding to the elution of a target analyte and of its labeled standards, the signal-to-noise ratio (S/N) must be better than or equal to 10. Measurement of S/N is required for any GC peak that has an apparent S/N of less than 5:1. The result of the calculation must appear on the SICP above the GC peak in question.

7.7.1.4.4 Referring to Table 9, calculate the 17 relative response factors (RF) for unlabeled target analytes [RF(n); n = 1 to 17] relative to their appropriate internal standards (Table 5) and the nine RFs for the $^{13}C_{12}$-labeled internal standards [RF(is); is = 18 to 26)] relative to the two recovery standards (Table 5) according to the following formulae:

$$RF_n = \frac{(A_n^1 + A_n^2) \times Q_{is}}{(A_{is}^1 + A_{is}^2) \times Q_n}$$

$$RF_{is} = \frac{(A_{is}^1 + A_{is}^2) \times Q_{rs}}{(A_{rs}^1 + A_{rs}^2) \times Q_{is}}$$

where:

$A_n^1$ and $A_n^2$ = sum of the integrated ion abundances of the quantitation ions (Tables 6 and 9) for unlabeled PCDDs/PCDFs,

$A_{is}^1$ and $A_{is}^2$ = sum of the integrated ion abundances of the quantitation ions (Tables 6 and 9) for the labeled internal standards,

$A_{rs}^1$ and $A_{rs}^2$ = sum of the integrated ion abundances of the quantitation ions (Tables 6 and 9) for the labeled recovery standards,

$Q_{is}$ = quantity of the internal standard injected (pg),

$Q_{rs}$ = quantity of the recovery standard injected (pg), and

$Q_n$ = quantity of the unlabeled PCDD/PCDF analyte injected (pg).

The $RF_n$ and $RF_{is}$ are dimensionless quantities; the units used to express $Q_{is}$, $Q_{rs}$ and $Q_n$ must be the same.

7.7.1.4.5 Calculate the $\overline{RF}$ values and their respective percent relative standard deviations (%RSD) for the five calibration solutions:

$$\overline{RF}_n = \frac{\sum_{j=1}^{5} RF_{n(j)}}{5}$$

where n represents a particular PCDD/PCDF (2,3,7,8-substituted) congener (n = 1 to 17; Table 9), and j is the injection number (or calibration solution number; j = 1 to 5).

7.7.1.4.6 The relative response factors to be used for the determination of the concentration of total isomers in a homologous series (Table 9) are calculated as follows:

7.7.1.4.6.1 For congeners that belong to a homologous series containing only one isomer (e.g., OCDD and OCDF) or only one 2,3,7,8-substituted isomer (Table 4; TCDD, PeCDD, HpCDD, and TCDF), the $\overline{RF}$ used will be the same as the $\overline{RF}$ determined in Sec. 7.7.1.4.5.

NOTE: The calibration solutions do not contain $^{13}C_{12}$-OCDF as an internal standard. This is because a minimum resolving power of 12,000 is required to resolve the $[M+6]^+$ ion of $^{13}C_{12}$-OCDF from the $[M+2]^+$ ion of OCDD (and $[M+4]^+$ from $^{13}C_{12}$-OCDF with $[M]^+$ of OCDD). Therefore, the $\overline{RF}$ or OCDF is calculated relative to $^{13}C_{12}$-OCDD.

7.7.1.4.6.2 For congeners that belong to a homologous series containing more than one 2,3,7,8-substituted isomer (Table 4), the $\overline{RF}$ used for those homologous series will be the mean of the RFs calculated for all individual 2,3,7,8-substituted congeners using the equation below:

$$\overline{RF}_k = \frac{\sum_{n=1}^{t} RF_n}{t}$$

where:

k = 27 to 30 (Table 9), with 27 = PeCDF; 28 = HxCDF; 29 = HxCDD; and 30 = HpCDF,

t = total number of 2,3,7,8-substituted isomers present in the calibration solutions (Table 5) for each homologous series (e.g., two for PeCDF, four for HxCDF, three for HxCDD, two for HpCDF).

NOTE: Presumably, the HRGC/HRMS response factors of different isomers within a homologous series are different. However, this analytical protocol will make the assumption that the HRGC/HRMS responses of all isomers in a homologous series that do not have the 2,3,7,8-substitution pattern are the same as the responses of one or more of the 2,3,7,8-substituted isomer(s) in that homologous series.

7.7.1.4.7 Relative response factors ($RF_m$) to be used for the determination of the percent recoveries for the nine internal standards are calculated as follows:

$$RF_m = \frac{A_{is}^{m} \times Q_{rs}}{Q_{is}^{m} \times A_{rs}}$$

$$\overline{RF}_m = \frac{\sum_{j=1}^{5} RF_{m(j)}}{5}$$

where:

m = 18 to 26 (congener type) and j = 1 to 5 (injection number),

$A_{is}^{m}$ = sum of the integrated ion abundances of the quantitation ions (Tables 6 and 9) for a given internal standard (m = 18 to 26),

$A_{rs}$ = sum of the integrated ion abundances of the quantitation ions (Tables 6 and 9) for the appropriate recovery standard (see Table 5, footnotes),

$Q_{rs}$, $Q_{is}^{m}$ = quantities of, respectively, the recovery standard (rs) and a particular internal standard (is = m) injected (pg),

$RF_m$ = relative response factor of a particular internal standard (m) relative to an appropriate recovery standard, as determined from one injection, and

$\overline{RF}_m$ = calculated mean relative response factor of a particular internal standard (m) relative to an appropriate recovery standard, as determined from the five initial calibration injections (j).

7.7.2 Criteria for acceptable calibration - The criteria listed below for acceptable calibration must be met before sample analyses are performed.

7.7.2.1 The percent relative standard deviations for the mean response factors ($\overline{RF}_n$ and $\overline{RF}_m$) from the 17 unlabeled standards must not exceed ± 20 percent, and those for the nine labeled reference compounds must not exceed ± 30 percent.

7.7.2.2 The S/N for the GC signals present in every SICP (including the ones for the labeled standards) must be $\geq$ 10.

7.7.2.3 The ion abundance ratios (Table 8) must be within the specified control limits.

NOTE: If the criterion for acceptable calibration listed in Sec. 7.7.2.1 is met, the analyte-specific RF can then be considered independent of the analyte quantity for the calibration concentration range. The mean RFs will be used for all calculations until the routine calibration criteria (Sec. 7.7.4) are no longer met. At such time, new $\overline{RF}$ values will be calculated from a new set of injections of the calibration solutions.

7.7.3 Routine calibration (continuing calibration check) - Routine calibrations must be performed at the beginning of a 12-hour period, after successful mass resolution and GC resolution performance checks. A routine calibration is also required at the end of a 12-hour shift. Inject 2 µL of the concentration calibration solution HRCC-3 standard (Table 5). By using the same HRGC/HRMS conditions as used in Secs. 7.6.1 and 7.6.2, determine and document an acceptable calibration as provided in Sec. 7.7.4.

7.7.4 Criteria for acceptable routine calibration - The following criteria must be met before further analysis is performed.

7.7.4.1 The measured RFs [$RF_n$ for the unlabeled standards] obtained during the routine calibration runs must be within ± 20 percent of the mean values established during the initial calibration (Sec. 7.7.1.4.5).

7.7.4.2 The measured RFs [$RF_m$ for the labeled standards] obtained during the routine calibration runs must be within ± 30 percent of the mean values established during the initial calibration (Sec. 7.7.1.4.7).

7.7.4.3 The ion abundance ratios (Table 8) must be within the allowed control limits.

7.7.4.4 If either one of the criteria in Secs. 7.7.4.1 and 7.7.4.2 is not satisfied, repeat one more time. If these criteria are still not satisfied, the entire routine calibration process (Sec. 7.7.1) must be reviewed. If the ion abundance ratio criterion (Sec. 7.7.4.3) is not satisfied, refer to the note in Sec. 7.7.1.4.2 for resolution.

NOTE: An initial calibration must be carried out whenever the HRCC-3, the sample fortification, or the recovery standard solution is replaced by a new solution from a different lot.

7.8 Analysis

7.8.1 Remove the sample or blank extract (from Sec. 7.5.3.6) from storage. With a stream of dry, purified nitrogen, reduce the extract volume to 10 µL to 50 µL.

NOTE: A final volume of 20 µL or more should be used whenever possible. A 10-µL final volume is difficult to handle, and injection of 2 µL out of 10 µL leaves little sample for confirmations and repeat injections, and for archiving.

7.8.2 Inject a 2-µL aliquot of the extract into the GC, operated under the conditions that have been established to produce acceptable results with the performance check solution (Secs. 7.6.1 and 7.6.2).

7.8.3 Acquire SIM data according to Secs. 7.6.2 and 7.6.3. Use the same acquisition and mass spectrometer operating conditions previously used to determine the relative response factors (Secs. 7.7.1.4.4 through 7.7.1.4.7). Ions characteristic of polychlorinated diphenyl ethers are included in the descriptors listed in Table 6.

NOTE: The acquisition period must at least encompass the PCDD/PCDF overall retention time window previously determined (Sec. 8.2.1.3). Selected ion current profiles (SICP) for the lock-mass ions (one per mass descriptor) must also be recorded and included in the data package. These SICPs must be true representations of the evolution of the lock-mass ions amplitudes during the HRGC/HRMS run (see Sec. 8.2.2 for the proper level of reference compound to be metered into the ion chamber.) The analyst may be required to monitor a PFK ion, not as a lock-mass, but as a regular ion, in order to meet this requirement. It is recommended to examine the lock-mass ion SICP for obvious basic sensitivity and stability changes of the instrument during the GC/MS run that could affect the measurements.

7.8.4 Identification criteria - For a gas chromatographic peak to be identified as a PCDD or PCDF, it must meet all of the following criteria:

7.8.4.1 Retention times

7.8.4.1.1 For 2,3,7,8-substituted congeners, which have an isotopically-labeled internal or recovery standard present in the sample extract (this represents a total of 10 congeners including OCDD; Tables 2 and 3), the retention time (RRT; at maximum peak height) of the sample components (i.e., the two ions used for quantitation purposes listed in Table 6) must be within -1 to +3 seconds of the isotopically-labeled standard.

7.8.4.1.2 For 2,3,7,8-substituted compounds that do not have an isotopically-labeled internal standard present in the sample extract (this represents a total of six congeners; Table 3), the retention time must fall within 0.005 retention time units of the relative retention times measured in the routine calibration. Identification of OCDF is based on its retention time relative to $^{13}C_{12}$-OCDD as determined from the daily routine calibration results.

7.8.4.1.3 For non-2,3,7,8-substituted compounds (tetra through octa; totaling 119 congeners), the retention time must be within the corresponding homologous retention time windows established by analyzing the column performance check solution (Sec. 8.1.3).

7.8.4.1.4 The ion current responses for both ions used for quantitative purposes (e.g., for TCDDs: m/z 319.8965 and 321.8936) must reach maximum simultaneously (± 2 seconds).

7.8.4.1.5 The ion current responses for both ions used for the labeled standards (e.g., for $^{13}C_{12}$-TCDD: m/z 331.9368 and m/z 333.9339) must reach maximum simultaneously (± 2 seconds).

NOTE: The analyst is required to verify the presence of 1,2,8,9-TCDD and 1,3,4,6,8-PeCDF (Sec. 8.1.3) in the SICPs of the daily performance checks. Should either one compound be missing, the analyst is required to take corrective action as it may indicate a potential problem with the ability to detect all the PCDDs/PCDFs.

7.8.4.2 Ion abundance ratios

The integrated ion currents for the two ions used for quantitation purposes must have a ratio between the lower and upper limits established for the homologous series to which the peak is assigned. See Secs. 7.7.1.4.1 and 7.7.1.4.2 and Table 8 for details.

7.8.4.3 Signal-to-noise ratio

All ion current intensities must be $\geq$ 2.5 times noise level for positive identification of an unlabeled PCDD/PCDF compound or a group of coeluting isomers. Figure 6 describes the procedure to be followed for the determination of the S/N. Labeled analytes must have a S/N $\geq$ 10.

7.8.4.4 Polychlorinated diphenyl ether interferences

In addition to the above criteria, the identification of a GC peak as a PCDF can only be made if no signal having a S/N $\geq$ 2.5 is detected at the same retention time (± 2 seconds) in the corresponding polychlorinated diphenyl ether (PCDPE, Table 6) channel.

7.9 Calculations

7.9.1 For gas chromatographic peaks that have met the criteria outlined in Sec. 7.8.4, calculate the concentration of the PCDD or PCDF compounds using the formula:

$$C_x = \frac{A_x \times Q_{is}}{A_{is} \times W \times \overline{RF}_n}$$

where:

$C_x$ = concentration of unlabeled PCDD/PCDF congeners (or group of coeluting isomers within an homologous series) in pg/g,

$A_x$ = sum of the integrated ion abundances of the quantitation ions (Table 6) for unlabeled PCDDs/PCDFs,

$A_{is}$ = sum of the integrated ion abundances of the quantitation ions (Table 6) for the labeled internal standards,

$Q_{is}$ = quantity, in pg, of the internal standard added to the sample before extraction,

W = weight, in g, of the sample (solid or organic liquid), or volume in mL of an aqueous sample, and

$\overline{RF}_n$ = calculated mean relative response factor for the analyte ($\overline{RF}_n$ with n = 1 to 17; Sec. 7.7.1.4.5).

If the analyte is identified as one of the 2,3,7,8-substituted PCDDs or PCDFs, $\overline{RF}_n$ is the value calculated using the equation in Sec. 7.7.1.4.5. However, if it is a non-2,3,7,8-substituted congener, the $\overline{RF}(k)$ value is the one calculated using the equation in Sec. 7.7.1.4.6.2. ($RF_k$, for k = 27 to 30).

7.9.2 Calculate the percent recovery of the nine internal standards measured in the sample extract, using the formula:

$$\text{percent recovery} = \frac{A_{is} \times Q_{rs}}{Q_{is} \times A_{rs} \times \overline{RF}_n} \times 100$$

where:

$A_{is}$ = sum of the integrated ion abundances of the quantitation ions (Table 6) for the labeled internal standard,

$A_{rs}$ = sum of the integrated ion abundances of the quantitation ions (Table 6) for the labeled recovery standard; the selection of the recovery standard depends on the type of congeners (see Table 5, footnotes),

$Q_{is}$ = quantity, in pg, of the internal standard added to the sample before extraction,

$Q_{rs}$ = quantity, in pg, of the recovery standard added to the cleaned-up sample residue before HRGC/HRMS analysis, and

$\overline{RF}_m$ = calculated mean relative response factor for the labeled internal standard relative to the appropriate (see Table 5, footnotes) recovery standard. This represents the mean obtained in Sec. 7.7.1.4.7 ($\overline{RF}_m$ with m = 18 to 26).

NOTE: For human adipose tissue, adjust the percent recoveries by adding 1 percent to the calculated value to compensate for the 1 percent of the extract diverted for the lipid determination.

7.9.3 If the concentration in the final extract of any of the fifteen 2,3,7,8-substituted PCDD/PCDF compounds (Table 3) exceeds the upper method calibration limits (MCL) listed in Table 1 (e.g., 200 pg/µL for TCDD in soil), the linear range of response versus concentration may have been exceeded, and a second analysis of the sample (using a one-tenth aliquot) should be undertaken. The volumes of the internal and recovery standard solutions should remain the same as described for the sample preparation (Secs. 7.1 to 7.9.3).

If a smaller sample size would not be representative of the entire sample, one of the following options is recommended:

(1) Re-extract an additional aliquot of sufficient size to insure that it is representative of the entire sample. Spike it with a higher concentration of internal standard. Prior to GC/MS analysis, dilute the sample so that it has a concentration of internal standard equivalent to that present in the calibration standard. Then, analyze the diluted extract.

(2) Re-extract an additional aliquot of sufficient size to insure that it is representative of the entire sample. Spike it with a higher concentration of internal standard. Immediately following extraction, transfer the sample to a volumetric flask and dilute to known volume. Remove an appropriate aliquot and proceed with cleanup and analysis.

(3) Use the original analysis data to quantitate the internal standard recoveries. Respike the original extract (note that no additional cleanup is necessary) with 100 times the usual quantity of internal standards. Dilute the re-spiked extract by a factor of 100. Reanalyze the diluted sample using the internal standard recoveries calculated from the initial analysis to correct the results for losses during isolation and cleanup.

7.9.4 The total concentration for each homologous series of PCDD and PCDF is calculated by summing up the concentrations of all positively identified isomers of each homologous series. Therefore, the total should also include the 2,3,7,8-substituted congeners. The total number of GC signals included in the homologous total concentration value must be specified in the report. If an isomer is not detected, use zero (0) in this calculation.

7.9.5 Sample specific estimated detection limit - The sample specific estimated detection limit (EDL) is the concentration of a given analyte required to produce a signal with a peak height of at least 2.5 times the background signal level. An EDL is calculated for each 2,3,7,8-substituted congener that is not identified, regardless of whether or not other non-2,3,7,8-substituted isomers are present. Two methods of calculation can be used, as follows, depending on the type of response produced during the analysis of a particular sample.

7.9.5.1 Samples giving a response for both quantitation ions (Tables 6 and 9) that is less than 2.5 times the background level.

Use the expression below to calculate an EDL for each 2,3,7,8-substituted PCDD/PCDF that does not have a response with S/N $\geq$ 2.5). The background level is determined by measuring the range of the noise (peak to peak) for the two quantitation ions (Table 6) of a particular 2,3,7,8-substituted isomer within an homologous series, in the region of the SICP trace corresponding to the elution of the internal standard (if the congener possesses an internal standard) or in the region of the SICP where the congener is expected to elute by comparison with the routine calibration data (for those congeners that do not have a $^{13}C$-labeled standard), multiplying that noise height by 2.5, and relating the product to an estimated concentration that would produce that peak height. Use the formula:

$$EDL = \frac{2.5 \times H_x \times Q_{is}}{H_{is} \times W \times \overline{RF}_n}$$

where:

EDL = estimated detection limit for homologous 2,3,7,8-substituted PCDDs/PCDFs.

$H_x$ = sum of the height of the noise level for each quantitation ion (Table 6) for the unlabeled PCDDs/PCDFs, measured as shown in Figure 6.

$H_{is}$ = sum of the height of the noise level for each quantitation ion (Table 6) for the labeled internal standard, measured as shown in Figure 6.

W, $\overline{RF}_n$, and $Q_{is}$ retain the same meanings as defined in Sec. 7.9.1.

7.9.5.2 Estimated maximum possible concentration - An estimated maximum possible concentration (EMPC) is calculated for 2,3,7,8-substituted isomers that are characterized by a response with an S/N of at least 2.5 for both the quantitation ions, and meet all of the identification criteria in Sec. 7.8.4 except the ion abundance ratio criteria or when a peak representing a PCDPE has been detected. An EMPC is a worst-case estimate of the concentration. Calculate the EMPC according to the expression shown in Sec. 7.9.1.

7.9.6 The relative percent difference (RPD) of any duplicate sample results are calculated as follows:

$$RPD = \frac{|S_1 - S_2|}{\frac{S_1 + S_2}{2}} \times 100$$

where $S_1$ and $S_2$ represent sample and duplicate sample results.

7.9.7 The 2,3,7,8-TCDD toxicity equivalents (TE) of PCDDs and PCDFs present in the sample are calculated, if requested by the data user, according to the method recommended by the Chlorinated Dioxins Workgroup (CDWG) of the EPA and the Center for Disease Control (CDC). This method assigns a 2,3,7,8-TCDD toxicity equivalency factor (TEF) to each of the fifteen 2,3,7,8-substituted PCDDs and PCDFs (Table 3) and to OCDD and OCDF, as shown in Table 10. The 2,3,7,8-TCDD equivalent of the PCDDs and PCDFs present in the sample is calculated by summing the TEF times their concentration for each of the compounds or groups of compounds listed in Table 10. The exclusion of other homologous series such as mono-, di-, and tri- chlorinated dibenzodioxins and dibenzofurans does not mean that they are non-toxic. However, their toxicity, as known at this time, is much lower than the toxicity of the compounds listed in Table 10. The above procedure for calculating the 2,3,7,8-TCDD toxicity equivalents is not claimed by the CDWG to be based on a thoroughly established scientific foundation. The procedure, rather, represents a "consensus recommendation on science policy." Since the procedure may be changed in the future, reporting requirements for PCDD and PCDF data would still include the reporting of the analyte concentrations of the PCDD/PCDF congener as calculated in Secs. 7.9.1 and 7.9.4.

7.9.8 Two GC column TEF determination

7.9.8.1 The concentration of 2,3,7,8-TCDD (see note below), is calculated from the analysis of the sample extract on the 60-m DB-5 (or equivalent) fused-silica capillary column. The experimental conditions remain the same as the conditions described previously in Sec. 7.8, and the calculations are performed as outlined in Sec. 7.9. The chromatographic separation between the 2,3,7,8-TCDD and its close eluters (1,2,3,7/1,2,3,8-TCDD and 1,2,3,9-TCDD) must be equal or less than 25 percent valley.

7.9.8.2 The concentration of the 2,3,7,8-TCDF is obtained from the analysis of the sample extract on the 30-m DB-225 (or equivalent) fused-silica capillary column. However, the GC/MS conditions must be altered so that: (1) only the first three descriptors (i.e., tetra-, penta-, and hexachlorinated congeners) of Table 6 are used; and (2) the switching time between descriptor 2 (pentachlorinated congeners) and descriptor 3 (hexachlorinated congeners) takes place following the elution of $^{13}C_{12}$-1,2,3,7,8-PeCDD. The concentration calculations are performed as outlined in Sec. 7.9. The chromatographic separation between the 2,3,7,8-TCDF and its close eluters (2,3,4,7-TCDF and 1,2,3,9-TCDF) must be equal or less than 25 percent valley.

NOTE: The confirmation and quantitation of 2,3,7,8-TCDD (Sec. 7.9.7.1.1) may be accomplished on the SP-2330 GC column instead of the DB-5 column, provided the criteria listed in Sec. 8.2.1 are met and the requirements described in Sec. 8.3.2 are followed.

7.9.8.3 For a gas chromatographic peak to be identified as a 2,3,7,8-substituted PCDD/PCDF congener, it must meet the ion abundance and signal-to-noise ratio criteria listed in Secs. 7.8.4.2 and 7.8.4.3, respectively. In addition, the retention time identification criterion described in Sec. 7.8.4.1.1 applies here for congeners for which a carbon-labeled analogue is available in the sample extract. However, the relative retention time (RRT) of the 2,3,7,8-substituted congeners for which no carbon-labeled analogues are available must fall within 0.006 units of the carbon-labeled standard RRT. Experimentally, this is accomplished by using the attributions described in Table 11 and the results from the routine calibration run on the SP-2330 column.

## 8.0 QUALITY CONTROL

8.1 Refer to Chapter One for specific quality control (QC) procedures. Quality control to validate sample extraction is covered in Method 3500. If extract cleanup was performed, follow the QC in Method 3600 and in the specific cleanup method.

8.2 System performance criteria - System performance criteria are presented below. The laboratory may use the recommended GC column described in Sec. 4.2. It must be documented that all applicable system performance criteria (specified in Secs. 8.2.1 and 8.2.2) were met before analysis of any sample is performed. Sec. 7.6.1 provides recommended GC conditions that can be used to satisfy the required criteria. Figure 3 provides a typical 12-hour analysis sequence, whereby the response factors and mass spectrometer resolving power checks must be performed at the beginning and the end of each 12-hour period of operation. A GC column performance check is only required at the beginning of each 12-hour period during which samples are analyzed. An HRGC/HRMS method blank run is required between a calibration run and the first sample run. The same method blank extract may thus be analyzed more than once if the number of samples within a batch requires more than 12 hours of analyses.

8.2.1 GC column performance

8.2.1.1 Inject 2 µL (Sec. 4.1.1) of the column performance check solution (Sec. 5.7) and acquire selected ion monitoring (SIM) data as described in Sec. 7.6.2 within a total cycle time of $\leq$ 1 second (Sec. 7.6.3.1).

8.2.1.2 The chromatographic separation between 2,3,7,8-TCDD and the peaks representing any other unlabeled TCDD isomers must be resolved with a valley of $\leq$ 25 percent (Figure 4), where:

$$\text{Valley percent} = (x/y) \times (100)$$

x = measured as in Figure 4 from the 2,3,7,8-closest TCDD eluting isomer
y = the peak height of 2,3,7,8-TCDD

It is the responsibility of the laboratory to verify the conditions suitable for the appropriate resolution of 2,3,7,8-TCDD from all other TCDD isomers. The GC column performance check solution also contains the known first and last PCDD/PCDF eluters under the conditions specified in this protocol. Their retention times are used to determine the eight homologue retention time windows that are used for qualitative (Sec. 7.8.4.1) and quantitative purposes. All peaks (that includes $^{13}C_{12}$-2,3,7,8-TCDD) should be labeled and identified on the chromatograms. Furthermore, all first eluters of a homologous series should be labeled with the letter F, and all last eluters of a homologous series should be labeled with the letter L (Figure 4 shows an example of peak labeling for TCDD isomers). Any individual selected ion current profile (SICP) (for the tetras, this would be the SICP for m/z 322 and m/z 304) or the reconstructed homologue ion current (for the tetras, this would correspond to m/z 320 + m/z 322 + m/z 304 + m/z 306) constitutes an acceptable form of data presentation. An SICP for the labeled compounds (e.g., m/z 334 for labeled TCDD) is also required.

8.2.1.3 The retention times for the switching of SIM ions characteristic of one homologous series to the next higher homologous series must be indicated in the SICP. Accurate switching at the appropriate times is absolutely necessary for accurate

monitoring of these compounds. Allowable tolerance on the daily verification with the GC performance check solution should be better than 10 seconds for the absolute retention times of all the components of the mixture. Particular caution should be exercised for the switching time between the last tetrachlorinated congener (i.e., 1,2,8,9-TCDD) and the first pentachlorinated congener (i.e., 1,3,4,6,8-PeCDF), as these two compounds elute within 15 seconds of each other on the 60-m DB-5 column. A laboratory with a GC/MS system that is not capable of detecting both congeners (1,2,8,9-TCDD and 1,3,4,6,8-PeCDF) within one analysis must take corrective action. If the recommended column is not used, then the first-and last-eluting isomer of each homologue must be determined experimentally on the column which is used, and the appropriate isomers must then be used for window definition and switching times.

8.2.2 Mass spectrometer performance

8.2.2.1 The mass spectrometer must be operated in the electron ionization mode. A static resolving power of at least 10,000 (10 percent valley definition) must be demonstrated at appropriate masses before any analysis is performed (Sec. 7.8). Static resolving power checks must be performed at the beginning and at the end of each 12-hour period of operation. However, it is recommended that a check of the static resolution be made and documented before and after each analysis. Corrective action must be implemented whenever the resolving power does not meet the requirement.

8.2.2.2 Chromatography time for PCDDs and PCDFs exceeds the long term mass stability of the mass spectrometer. Because the instrument is operated in the high-resolution mode, mass drifts of a few ppm (e.g., 5 ppm in mass) can have serious adverse effects on instrument performance. Therefore, a mass drift correction is mandatory. To that effect, it is recommended to select a lock-mass ion from the reference compound (PFK is recommended) used for tuning the mass spectrometer. The selection of the lock-mass ion is dependent on the masses of the ions monitored within each descriptor. Table 6 offers some suggestions for the lock-mass ions. However, an acceptable lock-mass ion at any mass between the lightest and heaviest ion in each descriptor can be used to monitor and correct mass drifts. The level of the reference compound (PFK) metered into the ion chamber during HRGC/HRMS analyses should be adjusted so that the amplitude of the most intense selected lock-mass ion signal (regardless of the descriptor number) does not exceed 10 percent of the full scale deflection for a given set of detector parameters. Under those conditions, sensitivity changes that might occur during the analysis can be more effectively monitored.

NOTE: Excessive PFK (or any other reference substance) may cause noise problems and contamination of the ion source resulting in an increase in downtime for source cleaning.

8.2.2.3 Documentation of the instrument resolving power must then be accomplished by recording the peak profile of the high-mass reference signal (m/z 380.9760) obtained during the above peak matching experiment by using the low-mass PFK ion at m/z 304.9824 as a reference. The minimum resolving power of 10,000 must be demonstrated on the high-mass ion while it is transmitted at a lower accelerating voltage than the low-mass reference ion, which is transmitted at full sensitivity. The format of the peak profile representation (Figure 5) must allow manual determination of the resolution, i.e., the horizontal axis must be a calibrated mass scale

(amu or ppm per division). The result of the peak width measurement (performed at 5 percent of the maximum, which corresponds to the 10 percent valley definition) must appear on the hard copy and cannot exceed 100 ppm at m/z 380.9760 (or 0.038 amu at that particular mass).

8.3 Quality control samples

8.3.1 Performance evaluation samples - When available, performance evaluation (PE) samples containing known amounts of unlabeled 2,3,7,8-substituted PCDDs/PCDFs or other PCDD/PCDF congeners should be analyzed alongside routine field samples.

8.3.2 Performance check solutions

8.3.2.1 At the beginning of each 12-hour period during which samples are to be analyzed, an aliquot of the 1) GC column performance check solution and 2) high-resolution concentration calibration solution No. 3 (HRCC-3; see Table 5) shall be analyzed to demonstrate adequate GC resolution and sensitivity, response factor reproducibility, and mass range calibration, and to establish the PCDD/PCDF retention time windows. A mass resolution check shall also be performed to demonstrate adequate mass resolution using an appropriate reference compound (PFK is recommended). If the required criteria are not met, remedial action must be taken before any samples are analyzed.

8.3.2.2 To validate positive sample data, the routine or continuing calibration (HRCC-3; Table 5) and the mass resolution check must be performed also at the end of each 12-hour period during which samples are analyzed. Furthermore, an HRGC/HRMS method blank run must be recorded following a calibration run and the first sample run.

8.3.2.2.1 If the laboratory operates only during one period (shift) each day of 12 hours or less, the GC performance check solution must be analyzed only once (at the beginning of the period) to validate the data acquired during the period. However, the mass resolution and continuing calibration checks must be performed at the beginning as well as at the end of the period.

8.3.2.2.2 If the laboratory operates during consecutive 12-hour periods (shifts), analysis of the GC performance check solution must be performed at the beginning of each 12-hour period. The mass resolution and continuing calibration checks from the previous period can be used for the beginning of the next period.

8.3.2.3 Results of at least one analysis of the GC column performance check solution and of two mass resolution and continuing calibration checks must be reported with the sample data collected during a 12-hour period.

8.3.2.4 Deviations from criteria specified for the GC performance check or for the mass resolution check invalidate all positive sample data collected between analyses of the performance check solution, and the extracts from those positive samples shall be reanalyzed.

If the routine calibration run fails at the beginning of a 12-hour shift, the instructions in Sec. 7.7.4.4 must be followed. If the continuing calibration check performed at the end of a 12 hour period fails by no more than 25 percent RPD for the 17 unlabeled compounds and 35 percent RPD for the 9 labeled reference compounds, use the mean to the two "daily" RF values from the two daily routine calibration runs to compute the analyte concentrations, instead of the $\overline{RF}$ values obtained from the initial calibration. A new initial calibration (new $\overline{RF}$s) is required immediately (within two hours) following the analysis of the samples, whenever the RPD from the end-of-shift routine calibration exceeds 25 percent or 35 percent, respectively. Failure to perform a new initial calibration immediately following the analysis of the samples will automatically require reanalysis of all positive sample extracts analyzed before the failed end-of-shift continuing calibration check.

8.3.3 The GC column performance check mixture, high-resolution concentration calibration solutions, and the sample fortification solutions may be obtained from commercial sources.

8.3.4 Field blanks - Batches of field samples may contain a field blank sample of uncontaminated soil, sediment or water that is to be fortified before analysis according to Sec. 8.3.4.1. In addition to this field blank, a batch of samples may include a rinsate, which is a portion of the solvent that was used to rinse sampling equipment. The rinsate is analyzed to assure that the samples were not contaminated by the sampling equipment.

8.3.4.1 Fortified field blank

8.3.4.1.1 Weigh a 10-g portion or use 1-L (for aqueous samples) of the specified field blank sample and add 100 µL of the solution containing the nine internal standards (Table 2) diluted with 1.0 mL acetone (Sec. 7.1).

8.3.4.1.2 Extract by using the procedures beginning in Secs. 7.4.5 or 7.4.6, as applicable, add 10 µL of the recovery standard solution (Sec. 7.5.3.6) and analyze a 2-µL aliquot of the concentrated extract.

8.3.4.1.3 Calculate the concentration (Sec. 7.9.1) of 2,3,7,8-substituted PCDDs/PCDFs and the percent recovery of the internal standards (Sec. 7.9.2).

8.3.4.1.4 Extract and analyze a new simulated fortified field blank whenever new lots of solvents or reagents are used for sample extraction or for column chromatographic procedures.

8.3.4.2 Rinsate sample

8.3.4.2.1 Take a 100-mL (± 0.5 mL) portion of the sampling equipment rinse solvent (rinsate sample), filter, if necessary, and add 100 µL of the solution containing the nine internal standards (Table 2).

8.3.4.2.2 Using a K-D apparatus, concentrate to about 5 mL.

NOTE: As an option, a rotary evaporator may be used in place of the K-D apparatus for the concentration of the rinsate.

8.3.4.2.3 Transfer the 5 mL concentrate from the K-D concentrator tube in 1-mL portions to a 1-mL minivial, reducing the volume in the minivial as necessary with a gentle stream of dry nitrogen.

8.3.4.2.4 Rinse the K-D concentrator tube with two 0.5 mL portions of hexane and transfer the rinses to the 1 mL minivial. Concentrate with dry nitrogen, as necessary.

8.3.4.2.5 Just before analysis, add 10 µL recovery standard solution (Table 2) and reduce the volume to its final volume, as necessary (Sec. 7.8.1). No column chromatography is required.

8.3.4.2.6 Analyze an aliquot of the solution following the same procedures used to analyze samples.

8.3.4.2.7 Report percent recovery of the internal standard and the presence of any PCDD/PCDF compounds in µg/L of rinsate solvent.

8.3.5 Duplicate analyses

In each batch of samples, locate the sample specified for duplicate analysis, and analyze a second 10-g soil or sediment sample portion or 1-L water sample, or an appropriate amount of the type of matrix under consideration.

8.3.5.1 The results of the laboratory duplicates (percent recovery and concentrations of 2,3,7,8-substituted PCDD/PCDF compounds) should agree within 25 percent relative difference (difference expressed as percentage of the mean). Report all results.

8.3.5.2 Recommended actions to help locate problems

Verify satisfactory instrument performance (Secs. 8.2 and 8.3).

If possible, verify that no error was made while weighing the sample portions.

Review the analytical procedures with the performing laboratory personnel.

8.3.6 Matrix spike and matrix spike duplicate

8.3.6.1 Locate the sample for the MS and MSD analyses (the sample may be labeled "double volume").

8.3.6.2 Add an appropriate volume of the matrix spike fortification solution (Sec. 5.10) and of the sample fortification solution (Sec. 5.8), adjusting the fortification level as specified in Table 1 under IS Spiking Levels.

8.3.6.3 Analyze the MS and MSD samples as described in Sec. 7.

8.3.6.4 The results obtained from the MS and MSD samples (concentrations of 2,3,7,8-substituted PCDDs/PCDFs) should agree within 20 percent relative difference.

8.4 Percent recovery of the internal standards - For each sample, method blank and rinsate, calculate the percent recovery (Sec. 7.9.2). The percent recovery should be between 40 percent and 135 percent for all 2,3,7,8-substituted internal standards.

NOTE: A low or high percent recovery for a blank does not require discarding the analytical data but it may indicate a potential problem with future analytical data.

8.5 Identification criteria

8.5.1 If either one of the identification criteria appearing in Secs. 7.8.4.1.1 through 7.8.4.1.4 is not met for an homologous series, it is reported that the sample does not contain unlabeled 2,3,7,8-substituted PCDD/PCDF isomers for that homologous series at the calculated detection limit (Sec. 7.9.5)

8.5.2 If the first initial identification criteria (Secs. 7.8.4.1.1 through 7.8.4.1.4) are met, but the criteria appearing in Secs. 7.8.4.1.5 and 7.8.4.2 are not met, that sample is presumed to contain interfering contaminants. This must be noted on the analytical report form, and the sample should be rerun or the extract reanalyzed.

8.6 Unused portions of samples and sample extracts should be preserved for six months after sample receipt to allow further analyses.

8.7 Reuse of glassware is to be minimized to avoid the risk of contamination.

## 9.0 METHOD PERFORMANCE

9.1 Table 12 provides data from a comparison of Soxhlet extraction and pressurized fluid extraction of samples of ground chimney brick. The data are taken from Reference 8.

9.2 Table 13 provides data from a comparison of Soxhlet extraction and pressurized fluid extraction of samples of urban dust. The data are taken from Reference 8.

9.3 Table 14 provides data from a comparison of Soxhlet extraction and pressurized fluid extraction of samples of fly ash. PFE data are provided for samples that were pretreated with an HCl wash and for samples that were not pretreated, but were extracted with a mixture of toluene and acetic acid. The data are taken from Reference 8.

9.4 Table 15 provides data from a comparison of Soxhlet extraction and pressurized fluid extraction of a soil sample (EC-2) from the National Water Research Institute (Burlington, Ontario, Canada) that contains high levels of PCDDs and PCDFs. The data are taken from Reference 8.

9.5 Table 16 provides data from a comparison of Soxhlet extraction and pressurized fluid extraction of a sediment sample (HS-2) from the National Research Council Institute for Marine Biosciences (Halifax, Nova Scotia, Canada) that contains low levels of PCDDs and PCDFs. The data are taken from Reference 8.

9.6 Table 17 provides data from a comparison of Soxhlet extraction and pressurized fluid extraction for two field-contaminated sediment samples. The data are taken from Reference 8.

## 10.0 REFERENCES

1. "Control of Interferences in the Analysis of Human Adipose Tissue for 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin". D. G. Patterson, J.S. Holler, D.F. Grote, L.R. Alexander, C.R. Lapeza, R.C. O'Connor and J.A. Liddle. *Environ. Toxicol. Chem.* 5, 355-360 (1986).

2. "Method 8290: Analytical Procedures and Quality Assurance for Multimedia Analysis of Polychlorinated Dibenzo-*p*-Dioxins and Dibenzofurans by High-Resolution Gas Chromatography/High-Resolution Mass Spectrometry". Y. Tondeur and W.F. Beckert. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Las Vegas, NV.

3. "Carcinogens - Working with Carcinogens", Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control. National Institute for Occupational Safety and Health. Publication No. 77-206, August 1977.

4. "OSHA Safety and Health Standards, General Industry", (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206 (revised January 1976).

5. "Safety in Academic Chemistry Laboratories", American Chemical Society Publication, Committee on Chemical Safety (3rd Edition, 1979.)

6. "Hybrid HRGC/MS/MS Method for the Characterization of Tetrachlorinated Dibenzo-p-dioxins in Environmental Samples." Y. Tondeur, W.J. Niederhut, S.R. Missler, and J.E. Campana, *Mass Spectrom.* 14, 449-456 (1987).

7. USEPA National Dioxin Study - Phase II, "Analytical Procedures and Quality Assurance Plan for the Determination of PCDD/PCDF in Fish", EPA-Duluth, October 26, 1987.

8. "Extraction of Polychlorinated Dibenzo-p-Dioxins and Polychlorinated Dibenzofurans from Environmental Samples Using Accelerated Solvent Extraction (ASE)," B. E. Richter, J. L. Ezzell, D. E. Knowles, and F. Hoefler, *Chemosphere*, 34 (5-7), 975-987, 1997.

## 11.0 SAFETY

11.1 The following safety practices are excerpts from EPA Method 613, Sec. 4 (July 1982 version) and amended for use in conjunction with this method. The 2,3,7,8-TCDD isomer has been found to be acnegenic, carcinogenic, and teratogenic in laboratory animal studies. Other PCDDs and PCDFs containing chlorine atoms in positions 2,3,7,8 are known to have toxicities comparable to that of 2,3,7,8-TCDD. The analyst should note that finely divided dry soils contaminated with PCDDs and PCDFs are particularly hazardous because of the potential for inhalation and ingestion. It is recommended that such samples be processed in a confined environment, such as a hood or a glove box. Laboratory personnel handling these types of samples should wear masks fitted with charcoal filters to prevent inhalation of dust.

11.2 The toxicity or carcinogenicity of each reagent used in this method is not precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be kept to a minimum. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets should be made available to all personnel involved in the chemical analysis of samples suspected to contain PCDDs/PCDFs. Additional references to laboratory safety are given in references 3, 4 and 5.

11.3 Each laboratory must develop a strict safety program for the handling of PCDDs and PCDFs. The laboratory practices listed below are recommended.

11.3.1 Contamination of the laboratory will be minimized by conducting most of the manipulations in a hood.

11.3.2 The effluents of sample splitters for the gas chromatograph and roughing pumps on the HRGC/HRMS system should pass through either a column of activated charcoal or be bubbled through a trap containing oil or high boiling alcohols.

11.3.3 Liquid waste should be dissolved in methanol or ethanol and irradiated with ultraviolet light at a wavelength less than 290 nm for several days (use F 40 BL lamps, or equivalent). Using this analytical method, analyze the irradiated liquid wastes and dispose of the solutions when 2,3,7,8-TCDD and 2,3,7,8-TCDF congeners can no longer be detected.

11.4 The following precautions for safe handling of 2,3,7,8-TCDD in the laboratory were issued by Dow Chemical U.S.A. (revised 11/78) and amended for use in conjunction with this method. They are as complete as possible on the basis of available toxicological information. The precautions for safe handling and use are necessarily general in nature since detailed, specific recommendations can be made only for the particular exposure and circumstances of each individual use. Assistance in evaluating the health hazards of particular plant conditions may be obtained from certain consulting laboratories and from State Departments of Health or of Labor, many of which have an industrial health service. The 2,3,7,8-TCDD isomer is extremely toxic to certain kinds of laboratory animals. However, it has been handled for years without injury in analytical and biological laboratories. Many techniques used in handling radioactive and infectious materials are applicable to 2,3,7,8-TCDD.

11.4.1 Protective equipment: Throw away plastic gloves, apron or lab coat, safety glasses and laboratory hood adequate for radioactive work. However, PVC gloves should not be used.

11.4.2 Training: Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces.

11.4.3 Personal hygiene: Thorough washing of hands and forearms after each manipulation and before breaks (coffee, lunch, and shift).

11.4.4 Confinement: Isolated work area, posted with signs, segregated glassware and tools, plastic backed absorbent paper on benchtops.

11.4.5 Waste: Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans.

11.4.6 Disposal of hazardous wastes: Refer to the November 7, 1986 issue of the Federal Register on Land Ban Rulings for details concerning the handling of dioxin-containing wastes.

11.4.7 Personnel decontamination: Apply a mild soap with plenty of scrubbing action. Glassware, tools and surfaces - Chlorothene NU Solvent™ (Dow Chemical Company) is the least toxic solvent shown to be effective. Satisfactory cleaning may be accomplished by rinsing with Chlorothene, then washing with a detergent and water. Dishwater may be

disposed to the sewer after percolation through a charcoal bed filter. It is prudent to minimize solvent wastes because they require costly special disposal through commercial services.

11.4.8 Laundry: Clothing known to be contaminated should be disposed with the precautions described under "Disposal of Hazardous Wastes". Laboratory coats or other clothing worn in 2,3,7,8-TCDD work area may be laundered. Clothing should be collected in plastic bags. Persons who convey the bags and launder the clothing should be advised of the hazard and trained in proper handling. The clothing may be put into a washer without contact if the launderer knows the problem. The washer should be run through one full cycle before being used again for other clothing.

11.4.9 Wipe tests: A useful method for determining cleanliness of work surfaces and tools is to wipe the surface with a piece of filter paper, extract the filter paper and analyze the extract.

NOTE: A procedure for the collection, handling, analysis, and reporting requirements of wipe tests performed within the laboratory is described in Appendix A. The results and decision making processes are based on the presence of 2,3,7,8-substituted PCDDs/PCDFs.

11.4.10 Inhalation: Any procedure that may generate airborne contamination must be carried out with good ventilation. Gross losses to a ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no significant inhalation hazards except in case of an accident.

11.4.11 Accidents: Remove contaminated clothing immediately, taking precautions not to contaminate skin or other articles. Wash exposed skin vigorously and repeatedly until medical attention is obtained.

APPENDIX A

PROCEDURES FOR THE COLLECTION, HANDLING, ANALYSIS, AND REPORTING OF WIPE TESTS PERFORMED WITHIN THE LABORATORY

This procedure is designed for the periodic evaluation of potential contamination by 2,3,7,8-substituted PCDD/PCDF congeners of the working areas inside the laboratory.

A.1 Perform the wipe tests on surface areas of two inches by one foot with glass fiber paper saturated with distilled in glass acetone using a pair of clean stainless steel forceps. Use one wiper for each of the designated areas. Combine the wipers to one composite sample in an extraction jar containing 200 mL of distilled-in-glass acetone. Place an equal number of unused wipers in 200 mL acetone and use this as a control. Add 100 µL of the sample fortification solution (Sec. 5.8) to each jar containing used or unused wipers.

A.1.1 Close the jar containing the wipers and the acetone and extract for 20 minutes using a wrist action shaker. Transfer the extract into a K-D apparatus fitted with a concentration tube and a three-ball Snyder column. Add two PTFE or Carborundum ™ boiling chips and concentrate the extract to an apparent volume of 1.0 mL on a steam bath. Rinse the Snyder column and the K-D assembly with two 1-mL portions of hexane into the concentrator tube, and concentrate its contents to near dryness with a gentle stream of nitrogen. Add 1.0 mL of hexane to the concentrator tube and swirl the solvent on the walls.

A.1.2 Prepare a neutral alumina column as described in Sec. 7.5.2.2 and follow the steps outlined in Secs. 7.5.2.3 through 7.5.2.5.

A.1.3 Add 10 µL of the recovery standard solution as described in Sec. 7.5.3.6.

A.2 Concentrate the contents of the vial to a final volume of 10 µL (either in a minivial or in a capillary tube). Inject 2 µL of each extract (wipe and control) onto a capillary column and analyze for 2,3,7,8-substituted PCDDs/PCDFs as specified in the analytical method in Sec. 7.8. Perform calculations according to Sec. 7.9.

A.3 Report the presence of 2,3,7,8-substituted PCDDs and PCDFs as a quantity (pg or ng) per wipe test experiment (WTE). Under the conditions outlined in this analytical protocol, a lower limit of calibration of 10 pg/WTE is expected for 2,3,7,8-TCDD. A positive response for the blank (control) is defined as a signal in the TCDD retention time window at any of the masses monitored which is equivalent to or above 3 pg of 2,3,7,8-TCDD per WTE. For other congeners, use the multiplication factors listed in Table 1, footnote (a) (e.g., for OCDD, the lower MCL is 10 x 5 = 50 pg/WTE and the positive response for the blank would be 3 x 5 = 15 pg). Also, report the recoveries of the internal standards during the simplified cleanup procedure.

A.4 At a minimum, wipe tests should be performed when there is evidence of contamination in the method blanks.

A.5 An upper limit of 25 pg per TCDD isomer and per wipe test experiment is allowed (use multiplication factors listed in footnote (a) from Table 1 for other congeners). This value corresponds to 2½ times the lower calibration limit of the analytical method. Steps to correct the contamination must be taken whenever these levels are exceeded. To that effect, first vacuum the working places (hoods, benches, sink) using a vacuum cleaner equipped with a high efficiency particulate absorbent (HEPA) filter and then wash with a detergent. A new set of wipes should be analyzed before anyone is allowed to work in the dioxin area of the laboratory after corrective action has been taken.

TABLE 1

TYPES OF MATRICES, SAMPLE SIZES, AND 2,3,7,8-TCDD-BASED METHOD CALIBRATION LIMITS (PARTS PER TRILLION)

| | Water | Soil Sediment Paper Pulp[b] | Fly Ash | Fish Tissue[c] | Human Adipose Tissue | Sludge Fuel Oil | Still Bottom |
|---|---|---|---|---|---|---|---|
| Lower MCL[a] | 0.01 | 1.0 | 1.0 | 1.0 | 1.0 | 5.0 | 10 |
| Upper MCL[a] | 2 | 200 | 200 | 200 | 200 | 1000 | 2000 |
| Sample Weight (g) | 1000 | 10 | 10 | 20 | 10 | 2 | 1 |
| IS Spiking Level (ppt) | 1 | 100 | 100 | 100 | 100 | 500 | 1000 |
| Final Ext. Vol. (µL)[d] | 10-50 | 10-50 | 50 | 10-50 | 10-50 | 50 | 50 |

[a] For other congeners multiply the values by 1 for TCDF/PeCDD/PeCDF, by 2.5 for HxCDD/HxCDF/HpCDD/HpCDF, and by 5 for OCDD/OCDF.

[b] Sample dewatered according to Sec. 6.5.

[c] One half of the extract from the 20 g sample is used for determination of lipid content (Sec. 7.2.2).

[d] See Sec. 7.8.1.

NOTE: Chemical reactor residues are treated as still bottoms, if their appearances so suggest.

TABLE 2

COMPOSITION OF THE SAMPLE FORTIFICATION
AND RECOVERY STANDARD SOLUTIONS[a]

| Analyte | Sample Fortification Solution Concentration (pg/µL) | Recovery Standard Solution Concentration (pg/µL) |
|---|---|---|
| $^{13}C_{12}$-2,3,7,8-TCDD | 10 | -- |
| $^{13}C_{12}$-2,3,7,8-TCDF | 10 | -- |
| $^{13}C_{12}$-1,2,3,4-TCDD | -- | 50 |
| $^{13}C_{12}$-1,2,3,7,8-PeCDD | 10 | -- |
| $^{13}C_{12}$-1,2,3,7,8-PeCDF | 10 | -- |
| $^{13}C_{12}$-1,2,3,6,7,8-HxCDD | 25 | -- |
| $^{13}C_{12}$-1,2,3,4,7,8-HxCDF | 25 | -- |
| $^{13}C_{12}$-1,2,3,7,8,9-HxCDD | -- | 50 |
| $^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD | 25 | -- |
| $^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF | 25 | -- |
| $^{13}C_{12}$-OCDD | 50 | -- |

[a]These solutions should be made freshly every day in nonane or other appropriate solvent because of the possibility of adsorptive losses to glassware. If these solutions are to be kept for more than one day, then the sample fortification solution concentrations should be increased ten fold, and the recovery standard solution concentrations should be doubled. Corresponding adjustments of the spiking volumes must then be made.

TABLE 3

THE FIFTEEN 2,3,7,8-SUBSTITUTED PCDD AND PCDF CONGENERS

| PCDD | PCDF |
|---|---|
| 2,3,7,8-TCDD(*) | 2,3,7,8-TCDF(*) |
| 1,2,3,7,8-PeCDD(*) | 1,2,3,7,8-PeCDF(*) |
| 1,2,3,6,7,8-HxCDD(*) | 2,3,4,7,8-PeCDF |
| 1,2,3,4,7,8-HxCDD | 1,2,3,6,7,8-HxCDF |
| 1,2,3,7,8,9-HxCDD(+) | 1,2,3,7,8,9-HxCDF |
| 1,2,3,4,6,7,8-HpCDD(*) | 1,2,3,4,7,8-HxCDF(*) |
| | 2,3,4,6,7,8-HxCDF |
| | 1,2,3,4,6,7,8-HpCDF(*) |
| | 1,2,3,4,7,8,9-HpCDF |

* The 13C-labeled analogue is used as an internal standard.

+ The 13C-labeled analogue is used as a recovery standard.

TABLE 4

ISOMERS OF CHLORINATED DIOXINS AND FURANS
AS A FUNCTION OF THE NUMBER OF CHLORINE ATOMS

| Number of Chlorine Atoms | Number of Dioxin Isomers | Number of 2,3,7,8-Dioxins | Number of Furan Isomers | Number of 2,3,7,8-Furans |
|---|---|---|---|---|
| 1 | 2 | --- | 4 | --- |
| 2 | 10 | --- | 16 | --- |
| 3 | 14 | --- | 28 | --- |
| 4 | 22 | 1 | 38 | 1 |
| 5 | 14 | 1 | 28 | 2 |
| 6 | 10 | 3 | 16 | 4 |
| 7 | 2 | 1 | 4 | 2 |
| 8 | 1 | 1 | 1 | 1 |
| Total | 75 | 7 | 135 | 10 |

TABLE 5

HIGH-RESOLUTION CONCENTRATION CALIBRATION SOLUTIONS

| Analyte | Concentration (pg/µL) | | | | |
|---|---|---|---|---|---|
| Unlabeled Analytes | 5 | 4 | 3 | 2 | 1 |
| 2,3,7,8-TCDD | 200 | 50 | 10 | 2.5 | 1 |
| 2,3,7,8-TCDF | 200 | 50 | 10 | 2.5 | 1 |
| 1,2,3,7,8-PeCDD | 500 | 125 | 25 | 6.25 | 2.5 |
| 1,2,3,7,8-PeCDF | 500 | 125 | 25 | 6.25 | 2.5 |
| 2,3,4,7,8-PeCDF | 500 | 125 | 25 | 6.25 | 2.5 |
| 1,2,3,4,7,8-HxCDD | 500 | 125 | 25 | 6.25 | 2.5 |
| 1,2,3,6,7,8-HxCDD | 500 | 125 | 25 | 6.25 | 2.5 |
| 1,2,3,7,8,9-HxCDD | 500 | 125 | 25 | 6.25 | 2.5 |
| 1,2,3,4,7,8-HxCDF | 500 | 125 | 25 | 6.25 | 2.5 |
| 1,2,3,6,7,8-HxCDF | 500 | 125 | 25 | 6.25 | 2.5 |
| 1,2,3,7,8,9-HxCDF | 500 | 125 | 25 | 6.25 | 2.5 |
| 2,3,4,6,7,8-HxCDF | 500 | 125 | 25 | 6.25 | 2.5 |
| 1,2,3,4,6,7,8-HpCDD | 500 | 125 | 25 | 6.25 | 2.5 |
| 1,2,3,4,6,7,8-HpCDF | 500 | 125 | 25 | 6.25 | 2.5 |
| 1,2,3,4,7,8,9-HpCDF | 500 | 125 | 25 | 6.25 | 2.5 |
| OCDD | 1,000 | 250 | 50 | 12.5 | 5 |
| OCDF | 1,000 | 250 | 50 | 12.5 | 5 |
| Internal Standards | | | | | |
| $^{13}C_{12}$-2,3,7,8-TCDD | 50 | 50 | 50 | 50 | 50 |
| $^{13}C_{12}$-2,3,7,8-TCDF | 50 | 50 | 50 | 50 | 50 |
| $^{13}C_{12}$-1,2,3,7,8-PeCDD | 50 | 50 | 50 | 50 | 50 |
| $^{13}C_{12}$-1,2,3,7,8-PeCDF | 50 | 50 | 50 | 50 | 50 |
| $^{13}C_{12}$-1,2,3,6,7,8-HxCDD | 125 | 125 | 125 | 125 | 125 |
| $^{13}C_{12}$-1,2,3,4,7,8-HxCDF | 125 | 125 | 125 | 125 | 125 |
| $^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD | 125 | 125 | 125 | 125 | 125 |
| $^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF | 125 | 125 | 125 | 125 | 125 |
| $^{13}C_{12}$-OCDD | 250 | 250 | 250 | 250 | 250 |
| Recovery Standards | | | | | |
| $^{13}C_{12}$-1,2,3,4-TCDD | 50 | 50 | 50 | 50 | 50 |
| $^{13}C_{12}$-1,2,3,7,8,9-HxCDD | 125 | 125 | 125 | 125 | 125 |

TABLE 6

IONS MONITORED FOR HRGC/HRMS ANALYSIS OF PCDDS/PCDFS

| Descriptor | Accurate Mass[a] | Ion ID | Elemental Composition | Analyte |
|---|---|---|---|---|
| 1 | 303.9016 | M | $C_{12}H_4{}^{35}Cl_4O$ | TCDF |
| | 305.8987 | M+2 | $C_{12}H_4{}^{35}Cl_3{}^{37}ClO$ | TCDF |
| | 315.9419 | M | $^{13}C_{12}H_4{}^{35}Cl_4O$ | TCDF (S) |
| | 317.9389 | M+2 | $^{13}C_{12}H_4{}^{35}Cl_3{}^{37}ClO$ | TCDF (S) |
| | 319.8965 | M | $C_{12}H_4{}^{35}Cl_4O_2$ | TCDD |
| | 321.8936 | M+2 | $C_{12}H_4{}^{35}Cl_3{}^{37}ClO_2$ | TCDD |
| | 331.9368 | M | $^{13}C_{12}H_4{}^{35}Cl_4O_2$ | TCDD (S) |
| | 333.9338 | M+2 | $^{13}C_{12}H_4{}^{35}Cl_3{}^{37}ClO_2$ | TCDD (S) |
| | 375.8364 | M+2 | $C_{12}H_4{}^{35}Cl_5{}^{37}ClO$ | HxCDPE |
| | [354.9792] | LOCK | $C_9F_{13}$ | PFK |
| 2 | 339.8597 | M+2 | $C_{12}H_3{}^{35}Cl_4{}^{37}ClO$ | PeCDF |
| | 341.8567 | M+4 | $C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O$ | PeCDF |
| | 351.9000 | M+2 | $^{13}C_{12}H_3{}^{35}Cl_4{}^{37}ClO$ | PeCDF (S) |
| | 353.8970 | M+4 | $^{13}C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O$ | PeCDF (S) |
| | 355.8546 | M+2 | $C_{12}H_3{}^{35}Cl_4{}^{37}ClO_2$ | PeCDD |
| | 357.8516 | M+4 | $C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O_2$ | PeCDD |
| | 367.8949 | M+2 | $^{13}C_{12}H_3{}^{35}Cl_4{}^{37}ClO_2$ | PeCDD (S) |
| | 369.8919 | M+4 | $^{13}C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O_2$ | PeCDD (S) |
| | 409.7974 | M+2 | $C_{12}H_3{}^{35}Cl_6{}^{37}ClO$ | HpCDPE |
| | [354.9792] | LOCK | $C_9F_{13}$ | PFK |
| 3 | 373.8208 | M+2 | $C_{12}H_2{}^{35}Cl_5{}^{37}ClO$ | HxCDF |
| | 375.8178 | M+4 | $C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O$ | HxCDF |
| | 383.8639 | M | $^{13}C_{12}H_2{}^{35}Cl_6O$ | HxCDF (S) |
| | 385.8610 | M+2 | $^{13}C_{12}H_2{}^{35}Cl_5{}^{37}ClO$ | HxCDF (S) |
| | 389.8156 | M+2 | $C_{12}H_2{}^{35}Cl_5{}^{37}ClO_2$ | HxCDD |
| | 391.8127 | M+4 | $C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O_2$ | HxCDD |

TABLE 6
(continued)

| Descriptor | Accurate Mass[a] | Ion ID | Elemental Composition | Analyte |
|---|---|---|---|---|
| | 401.8559 | M+2 | $^{13}C_{12}H_2{}^{35}Cl_5{}^{37}ClO_2$ | HxCDD (S) |
| | 403.8529 | M+4 | $^{13}C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O_2$ | HxCDD (S) |
| | 445.7555 | M+4 | $C_{12}H_2{}^{35}Cl_6{}^{37}Cl_2O$ | OCDPE |
| | [430.9728] | LOCK | $C_9F_{17}$ | PFK |
| 4 | 407.7818 | M+2 | $C_{12}H^{35}Cl_6{}^{37}ClO$ | HpCDF |
| | 409.7788 | M+4 | $C_{12}H^{35}Cl_5{}^{37}Cl_2O$ | HpCDF |
| | 417.8250 | M | $^{13}C_{12}H^{35}Cl_7O$ | HpCDF (S) |
| | 419.8220 | M+2 | $^{13}C_{12}H^{35}Cl_6{}^{37}ClO$ | HpCDF |
| | 423.7767 | M+2 | $C_{12}H^{35}Cl_6{}^{37}ClO_2$ | HpCDD |
| | 425.7737 | M+4 | $C_{12}H^{35}Cl_5{}^{37}Cl_2O_2$ | HpCDD |
| | 435.8169 | M+2 | $^{13}C_{12}H^{35}Cl_6{}^{37}ClO_2$ | HpCDD (S) |
| | 437.8140 | M+4 | $^{13}C_{12}H^{35}Cl_5{}^{37}Cl_2O_2$ | HpCDD (S) |
| | 479.7165 | M+4 | $C_{12}H^{35}Cl_7{}^{37}Cl_2O$ | NCDPE |
| | [430.9728] | LOCK | $C_9F_{17}$ | PFK |
| 5 | 441.7428 | M+2 | $C_{12}{}^{35}Cl_7{}^{37}ClO$ | OCDF |
| | 443.7399 | M+4 | $C_{12}{}^{35}Cl_6{}^{37}Cl_2O$ | OCDF |
| | 457.7377 | M+2 | $C_{12}{}^{35}Cl_7{}^{37}ClO_2$ | OCDD |
| | 459.7348 | M+4 | $C_{12}{}^{35}Cl_6{}^{37}Cl_2O_2$ | OCDD |
| | 469.7780 | M+2 | $^{13}C_{12}{}^{35}Cl_7{}^{37}ClO_2$ | OCDD (S) |
| | 471.7750 | M+4 | $^{13}C_{12}{}^{35}Cl_6{}^{37}Cl_2O_2$ | OCDD (S) |
| | 513.6775 | M+4 | $C_{12}{}^{35}Cl_8{}^{37}Cl_2O$ | DCDPE |
| | [442.9728] | LOCK | $C_{10}F_{17}$ | PFK |

S = internal/recovery standard

[a] The following nuclidic masses were used:

| | | | | | |
|---|---|---|---|---|---|
| H | = | 1.007825 | O | = | 15.994915 |
| C | = | 12.000000 | $^{35}Cl$ | = | 34.968853 |
| $^{13}C$ | = | 13.003355 | $^{37}Cl$ | = | 36.965903 |
| F | = | 18.9984 | | | |

TABLE 7

PCDD AND PCDF CONGENERS PRESENT IN THE GC
PERFORMANCE EVALUATION SOLUTION AND USED FOR DEFINING
THE HOMOLOGUE GC RETENTION TIME WINDOWS ON A 60-M DB-5 COLUMN

| | PCDD Positional Isomer | | PCDF Positional Isomer | |
|---|---|---|---|---|
| # Chlorine Atoms | First Eluter | Last Eluter | First Eluter | Last Eluter |
| 4[a] | 1,3,6,8 | 1,2,8,9 | 1,3,6,8 | 1,2,8,9 |
| 5 | 1,2,4,6,8/1,2,4,7,9 | 1,2,3,8,9 | 1,3,4,6,8 | 1,2,3,8,9 |
| 6 | 1,2,4,6,7,9/1,2,4,6,8,9 | 1,2,3,4,6,7 | 1,2,3,4,6,8 | 1,2,3,4,8,9 |
| 7 | 1,2,3,4,6,7,9 | 1,2,3,4,6,7,8 | 1,2,3,4,6,7,8 | 1,2,3,4,7,8,9 |
| 8 | 1,2,3,4,6,7,8,9 | | 1,2,3,4,6,7,8,9 | |

[a]In addition to these two TCDD isomers, the 1,2,3,4-, 1,2,3,7-, 1,2,3,8-, 2,3,7,8-, $^{13}C_{12}$-2,3,7,8-, and 1,2,3,9-TCDD isomers must also be present as a check of column resolution.

TABLE 8

THEORETICAL ION ABUNDANCE RATIOS AND THEIR CONTROL LIMITS
FOR PCDDS AND PCDFS

| # Chlorine Atoms | Ion Type | Theoretical Abundance Ratio | Control Limits | |
|---|---|---|---|---|
| | | | Lower | Upper |
| 4 | M/M+2 | 0.77 | 0.65 | 0.89 |
| 5 | M+2/M+4 | 1.55 | 1.32 | 1.78 |
| 6 | M+2/M+4 | 1.24 | 1.05 | 1.43 |
| 6[(a)] | M/M+2 | 0.51 | 0.43 | 0.59 |
| 7[(b)] | M/M+2 | 0.44 | 0.37 | 0.51 |
| 7 | M+2/M+4 | 1.04 | 0.88 | 1.20 |
| 8 | M+2/M+4 | 0.89 | 0.76 | 1.02 |

[a]Used only for $^{13}$C-HxCDF (IS).

[b]Used only for $^{13}$C-HpCDF (IS).

TABLE 9

RELATIVE RESPONSE FACTOR [RF (NUMBER)] ATTRIBUTIONS

| Number | Specific Congener Name |
|---|---|
| 1 | 2,3,7,8-TCDD (and total TCDDs) |
| 2 | 2,3,7,8-TCDF (and total TCDFs) |
| 3 | 1,2,3,7,8-PeCDD (and total PeCDDs) |
| 4 | 1,2,3,7,8-PeCDF |
| 5 | 2,3,4,7,8-PeCDF |
| 6 | 1,2,3,4,7,8-HxCDD |
| 7 | 1,2,3,6,7,8-HxCDD |
| 8 | 1,2,3,7,8,9-HxCDD |
| 9 | 1,2,3,4,7,8-HxCDF |
| 10 | 1,2,3,6,7,8-HxCDF |
| 11 | 1,2,3,7,8,9-HxCDF |
| 12 | 2,3,4,6,7,8-HxCDF |
| 13 | 1,2,3,4,6,7,8-HpCDD (and total HpCDDs) |
| 14 | 1,2,3,4,6,7,8-HpCDF |
| 15 | 1,2,3,4,7,8,9-HpCDF |
| 16 | OCDD |
| 17 | OCDF |
| 18 | $^{13}C_{12}$-2,3,7,8-TCDD |
| 19 | $^{13}C_{12}$-2,3,7,8-TCDF |
| 20 | $^{13}C_{12}$-1,2,3,7,8-PeCDD |
| 21 | $^{13}C_{12}$-1,2,3,7,8-PeCDF |
| 22 | $^{13}C_{12}$-1,2,3,6,7,8-HxCDD |
| 23 | $^{13}C_{12}$-1,2,3,4,7,8-HxCDF |
| 24 | $^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD |
| 25 | $^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF |
| 26 | $^{13}C_{12}$-OCDD |
| 27 | Total PeCDFs |
| 28 | Total HxCDFs |
| 29 | Total HxCDDs |
| 30 | Total HpCDFs |

TABLE 10

2,3,7,8-TCDD TOXICITY EQUIVALENCY FACTORS (TEFS)
FOR THE POLYCHLORINATED DIBENZODIOXINS AND DIBENZOFURANS

| Analyte | TEF[a] |
|---|---|
| 2,3,7,8-TCDD | 1.00 |
| 1,2,3,7,8-PeCDD | 0.50 |
| 1,2,3,6,7,8-HxCDD | 0.10 |
| 1,2,3,7,8,9-HxCDD | 0.10 |
| 1,2,3,4,7,8-HxCDD | 0.10 |
| 1,2,3,4,6,7,8-HpCDD | 0.01 |
| 1,2,3,4,6,7,8,9-OCDD | 0.001 |
| 2,3,7,8-TCDF | 0.1 |
| 1,2,3,7,8-PeCDF | 0.05 |
| 2,3,4,7,8-PeCDF | 0.5 |
| 1,2,3,6,7,8-HxCDF | 0.1 |
| 1,2,3,7,8,9-HxCDF | 0.1 |
| 1,2,3,4,7,8-HxCDF | 0.1 |
| 2,3,4,6,7,8-HxCDF | 0.1 |
| 1,2,3,4,6,7,8-HpCDF | 0.01 |
| 1,2,3,4,7,8,9-HpCDF | 0.01 |
| 1,2,3,4,6,7,8,9-OCDF | 0.001 |

[a]Taken from "Interim Procedures for Estimating Risks Associated with Exposures to Mixtures of Chlorinated Dibenzo-*p*-Dioxin and -Dibenzofurans (CDDs and CDFs) and 1989 Update", (EPA/625/3-89/016, March 1989).

TABLE 11

ANALYTE RELATIVE RETENTION TIME REFERENCE ATTRIBUTIONS

| Analyte | Analyte RRT Reference[a] |
|---|---|
| 1,2,3,4,7,8-HxCDD | $^{13}C_{12}$-1,2,3,6,7,8-HxCDD |
| 1,2,3,6,7,8-HxCDF | $^{13}C_{12}$-1,2,3,4,7,8-HxCDF |
| 1,2,3,7,8,9-HxCDF | $^{13}C_{12}$-1,2,3,4,7,8-HxCDF |
| 2,3,4,6,7,8-HxCDF | $^{13}C_{12}$-1,2,3,4,7,8-HxCDF |

[a]The retention time of 2,3,4,7,8-PeCDF on the DB-5 column is measured relative to $^{13}C_{12}$-1,2,3,7,8-PeCDF and the retention time of 1,2,3,4,7,8,9-HpCDF relative to $^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF.

TABLE 12

COMPARISON OF SOXHLET AND PRESSURIZED FLUID EXTRACTION (PFE) FOR EXTRACTION OF GROUND CHIMNEY BRICK

| Analyte | Soxhlet (n=1) (ng/kg) | PFE (n=2)* (ng/kg) |
|---|---|---|
| 2,3,7,8-TCDD | 6 | 6 |
| 1,2,3,7,8-PeCDD | 52 | 57 |
| 1,2,3,4,7,8-HxCDD | 46 | 52 |
| 1,2,3,6,7,8-HxCDD | 120 | 130 |
| 1,2,3,7,9,9-HxCDD | 97 | 1000 |
| 1,2,3,4,6,7,8-HpCDD | 1000 | 820 |
| OCDD | 2900 | 2600 |
| 2,3,7,8-TCDF | 160 | 180 |
| 1,2,3,7,8 ( + 1,2,3,4,8)-PeCDF | 430 | 470 |
| 2,3,4,7,9-PeCDF | 390 | 390 |
| 1,2,3,4,7,8 ( + 1,2,3,4,7,9)-HxCDF | 1100 | 1100 |
| 1,2,3,6,7,8-HxCDF | 540 | 570 |
| 2,3,4,6,7,8-HxCDF | 400 | 360 |
| 1,2,3,7,8,9-HxCDF | 42 | 42 |
| 1,2,3,4,6,7,8-HpCDF | 2100 | 2000 |
| 1,2,3,4,7,8,9-HpCDF | 140 | 120 |
| OCDF | 2000 | 2000 |
| Total TCDD | 440 | 530 |
| Total PeCDD | 900 | 940 |
| Total HxCDD | 1800 | 2000 |
| Total HpCDD | 2000 | 2100 |
| Total TCDF | 2300 | 2600 |
| Total PeCDF | 4100 | 4300 |
| Total HxCDF | 4700 | 4700 |
| Total HpCDF | 2800 | 2600 |

* Sum of two extractions of each sample
Data from Reference 8

TABLE 13

COMPARISON OF SOXHLET AND PFE FOR EXTRACTION OF URBAN DUST

| Analyte | Soxhlet (n=1) (ng/kg) | PFE (n=2)* (ng/kg) |
|---|---|---|
| 2,3,7,8-TCDD | 3.3 | 3.2 |
| 1,2,3,7,8-PeCDD | 11.8 | 13.1 |
| 1,2,3,4,7,8-HxCDD | 9.8 | 8.0 |
| 1,2,3,6,7,8-HxCDD | 11.5 | 9.5 |
| 1,2,3,7,9,9-HxCDD | ND (8) | ND (8) |
| 1,2,3,4,6,7,8-HpCDD | 113 | 107 |
| OCDD | 445 | 314 |
| 2,3,7,8-TCDF | 12.5 | 18.6 |
| 1,2,3,7,8 ( + 1,2,3,4,8)-PeCDF | 9.9 | 12.0 |
| 2,3,4,7,9-PeCDF | 13.9 | 18.1 |
| 1,2,3,4,7,8 ( + 1,2,3,4,7,9)-HxCDF | 18.7 | 23.7 |
| 1,2,3,6,7,8-HxCDF | 10.7 | 15.8 |
| 2,3,4,6,7,8-HxCDF | 3.3 | 8.7 |
| 1,2,3,7,8,9-HxCDF | ND (2) | ND (2) |
| 1,2,3,4,6,7,8-HpCDF | 13.2 | 29.4 |
| 1,2,3,4,7,8,9-HpCDF | ND (3) | ND (3) |
| OCDF | ND (10) | ND (10) |
| Total TCDD | 182 | 325 |
| Total PeCDD | 175 | 281 |
| Total HxCDD | 86.7 | 81.7 |
| Total HpCDD | 221 | 217 |
| Total TCDF | 333 | 419 |
| Total PeCDF | 146 | 179 |
| Total HxCDF | 65.9 | 122 |
| Total HpCDF | 13.2 | 29.4 |

ND = Not detected, with detection limit given in parentheses
* Sum of two extractions of each sample
Data from Reference 8

TABLE 14

COMPARISON OF SOXHLET AND PFE FOR EXTRACTION OF FLY ASH
(with and without HCl pretreatment for PFE)

| Analyte | Soxhlet (n=1) with HCl (µg/kg)† | PFE (n=2)* with HCl (µg/kg)† | PFE (n=2)* w/o HCl (µg/kg)‡ |
|---|---|---|---|
| 2,3,7,8-TCDD | 0.32 | 0.36 | 0.28 |
| 1,2,3,7,8-PeCDD | 1.6 | 2.1 | 1.7 |
| 1,2,3,4,7,8-HxCDD | 1.2 | 1.4 | 1.2 |
| 1,2,3,6,7,8-HxCDD | 2.4 | 2.7 | 2.4 |
| 1,2,3,7,9,9-HxCDD | 2.4 | 2.3 | 2.2 |
| 1,2,3,4,6,7,8-HpCDD | 8.2 | 9.6 | 8.1 |
| OCDD | 11.4 | 12.8 | 10.6 |
| 2,3,7,8-TCDF | 3.7 | 4.3 | 3.4 |
| 1,2,3,7,8 ( + 1,2,3,4,8)-PeCDF | 4.2 | 4.6 | 3.9 |
| 2,3,4,7,9-PeCDF | 5.6 | 6.6 | 5.8 |
| 1,2,3,4,7,8 ( + 1,2,3,4,7,9)-HxCDF | 7.8 | 8.7 | 5.4 |
| 1,2,3,6,7,8-HxCDF | 7.2 | 8.5 | 5.3 |
| 2,3,4,6,7,8-HxCDF | 6.6 | 7.2 | 4.5 |
| 1,2,3,7,8,9-HxCDF | 0.43 | 0.56 | 0.30 |
| 1,2,3,4,6,7,8-HpCDF | 18.0 | 17.6 | 16.8 |
| 1,2,3,4,7,8,9-HpCDF | 2.3 | 2.4 | 2.0 |
| OCDF | 13.5 | 15.8 | 13.9 |
| Total TCDD | 12.0 | 12.4 | 10.5 |
| Total PeCDD | 16.6 | 20.5 | 16.2 |
| Total HxCDD | 38.2 | 42.4 | 36.7 |
| Total HpCDD | 15.0 | 19.8 | 16.0 |
| Total TCDF | 60.5 | 67.5 | 56.1 |
| Total PeCDF | 83.5 | 87.3 | 77.4 |
| Total HxCDF | 65.2 | 73.5 | 46.1 |
| Total HpCDF | 28.1 | 32.2 | 26.5 |

† Fly ash was pretreated with HCl, followed by a water rinse, and extracted with toluene.
‡ These samples received no HCl pretreatment, and were extracted with a mixture of toluene and acetic acid.
* Sum of two extractions of each sample
Data from Reference 8

TABLE 15

COMPARISON OF SOXHLET AND PFE FOR EXTRACTION OF SOIL (EC-2)

| | Soxhlet Results (n=10) | | PFE Results (n=2) | |
|---|---|---|---|---|
| Analyte | ng/kg | % RSD | ng/kg | % RSD |
| 2,3,7,8-TCDD | 270 | 9.1 | 270 | 0.0 |
| 1,2,3,7,8-PeCDD | 24 | 12 | 22 | 3.3 |
| 1,2,3,4,7,8-HxCDD | 23 | 8.3 | 24 | 3.0 |
| 1,2,3,6,7,8-HxCDD | 83 | 3.6 | 87 | 0.8 |
| 1,2,3,7,9,9-HxCDD | 60 | 6.2 | 57 | 7.4 |
| 1,2,3,4,6,7,8-HpCDD | 720 | 6.7 | 720 | 1.0 |
| OCDD | 4000 | 6.2 | 4200 | 0.0 |
| 2,3,7,8-TCDF * | 100 | 7.3 | 82 | 2.6 |
| 1,2,3,7,8 ( + 1,2,3,4,8)-PeCDF | 39 | 14 | 36 | 3.9 |
| 2,3,4,7,9-PeCDF | 62 | 5.5 | 60 | 0.0 |
| 1,2,3,4,7,8 ( + 1,2,3,4,7,9)-HxCDF | 740 | 5.3 | 690 | 0.0 |
| 1,2,3,6,7,8-HxCDF | 120 | 6.2 | 120 | 0.0 |
| 2,3,4,6,7,8-HxCDF | 45 | 9.0 | 60 | 1.2 |
| 1,2,3,7,8,9-HxCDF | 4.9 | 31 | 5.3 | 15 |
| 1,2,3,4,6,7,8-HpCDF | 2600 | 6.7 | 2500 | 0.0 |
| 1,2,3,4,7,8,9-HpCDF | 160 | 5.5 | 160 | 0.0 |
| OCDF | 7800 | 8.3 | 7000 | 3.1 |
| Total TCDD | 430 | 9.7 | 370 | 1.9 |
| Total PeCDD | 300 | 3.7 | 280 | 7.7 |
| Total HxCDD | 720 | 5.8 | 690 | 2.0 |
| Total HpCDD | 1300 | 7.0 | 1300 | 0.0 |
| Total TCDF | 620 | 12 | 380 | 19 |
| Total PeCDF | 820 | 9.4 | 710 | 7.0 |
| Total HxCDF | 1900 | 5.7 | 1900 | 0.0 |
| Total HpCDF | 3800 | 8.2 | 3900 | 3.6 |

* Single-column analysis only, may include contributions from other isomers that may co-elute.
Data from Reference 8

TABLE 16

COMPARISON OF SOXHLET AND PFE FOR EXTRACTION OF SEDIMENT (HS-2)

| | Soxhlet Results (n=10) | | PFE Results (n=2) | |
|---|---|---|---|---|
| Analyte | ng/kg | % RSD | ng/kg | % RSD |
| 2,3,7,8-TCDD | ND (1) | -- | ND (1) | -- |
| 1,2,3,7,8-PeCDD | 1.6 | 4.6 | ND (1) | -- |
| 1,2,3,4,7,8-HxCDD | 4.5 | 4.8 | 5.2 | 11 |
| 1,2,3,6,7,8-HxCDD | 19 | 4.3 | 21 | 0.0 |
| 1,2,3,7,9,9-HxCDD | 24 | 4.3 | 28 | 2.6 |
| 1,2,3,4,6,7,8-HpCDD | 1200 | 8.1 | 1300 | 0.0 |
| OCDD | 6500 | 4.2 | 7100 | 0.0 |
| 2,3,7,8-TCDF * | 8.5 | 11 | 6.6 | 5.4 |
| 1,2,3,7,8 ( + 1,2,3,4,8)-PeCDF | 1.9 | 17 | 2.0 | 0.0 |
| 2,3,4,7,9-PeCDF | 3.7 | 7.9 | 3.7 | 3.8 |
| 1,2,3,4,7,8 ( + 1,2,3,4,7,9)-HxCDF | 17 | 7.3 | 17 | 4.3 |
| 1,2,3,6,7,8-HxCDF | 3.7 | 5.6 | 4.0 | 5.4 |
| 2,3,4,6,7,8-HxCDF | 3.7 | 18 | 4.4 | 3.2 |
| 1,2,3,7,8,9-HxCDF | ND (1) | -- | ND (1) | -- |
| 1,2,3,4,6,7,8-HpCDF | 91 | 1.6 | 96 | 3.7 |
| 1,2,3,4,7,8,9-HpCDF | 5.2 | 6.7 | 5.3 | 6.7 |
| OCDF | 300 | 3.8 | 280 | 2.6 |
| Total TCDD | 3.9 | 14 | 2.5 | 34 |
| Total PeCDD | 17 | 7.8 | 10 | 10 |
| Total HxCDD | 510 | 5.6 | 570 | 1.3 |
| Total HpCDD | 4700 | 8.3 | 5100 | 11 |
| Total TCDF | 39 | 11 | 24 | 3.0 |
| Total PeCDF | 33 | 13 | 28 | 0.0 |
| Total HxCDF | 89 | 3.2 | 87 | 12 |
| Total HpCDF | 293 | 3.3 | 310 | 0.0 |

* Single-column analysis only, may include contributions from other isomers that may co-elute.
. ND = Not detected, with detection limit given in parentheses
. Data from Reference 8

TABLE 17

COMPARISON OF SOXHLET AND PFE FOR EXTRACTION OF CONTAMINATED SEDIMENTS

| | Hamilton Harbor | | Parrots Bay | |
|---|---|---|---|---|
| Analyte | Soxhlet | PFE | Soxhlet | PFE |
| 2,3,7,8-TCDD | 3.7 | 3.1 | 19 | 19 |
| 1,2,3,7,8-PeCDD | 5.1 | 5.4 | 8.3 | 6.0 |
| 1,2,3,4,7,8-HxCDD | 6.4 | 7.2 | 8.6 | 6.7 |
| 1,2,3,6,7,8-HxCDD | 27 | 26 | 26 | 17 |
| 1,2,3,7,9,9-HxCDD | 20 | 28 | 24 | 18 |
| 1,2,3,4,6,7,8-HpCDD | 460 | 430 | 280 | 250 |
| OCDD | 3100 | 3100 | 1900 | 1600 |
| 2,3,7,8-TCDF * | 61 | 44 | 80 | 48 |
| 1,2,3,7,8 ( + 1,2,3,4,8)-PeCDF | 14 | 14 | ND (20) | 9.8 |
| 2,3,4,7,9-PeCDF | 26 | 25 | 22 | 14 |
| 1,2,3,4,7,8 ( + 1,2,3,4,7,9)-HxCDF | 27 | 37 | 79 | 59 |
| 1,2,3,6,7,8-HxCDF | 17 | 16 | ND (20) | 15 |
| 2,3,4,6,7,8-HxCDF | 14 | 14 | 21 | 11 |
| 1,2,3,7,8,9-HxCDF | ND (2) | 1.6 | 4.9 | ND (1) |
| 1,2,3,4,6,7,8-HpCDF | 130 | 130 | 270 | 220 |
| 1,2,3,4,7,8,9-HpCDF | 14 | 13 | 17 | 12 |
| OCDF | 270 | 210 | 510 | 370 |
| Total TCDD | 50 | 14 | 39 | 48 |
| Total PeCDD | 63 | 15 | 87 | 66 |
| Total HxCDD | 220 | 180 | 230 | 200 |
| Total HpCDD | 850 | 810 | 580 | 530 |
| Total TCDF | 370 | 130 | 400 | 270 |
| Total PeCDF | 290 | 110 | 180 | 170 |
| Total HxCDF | 240 | 160 | 230 | 230 |
| Total HpCDF | 350 | 290 | 400 | 360 |

* Single-column analysis only, may include contributions from other isomers that may co-elute.
. ND = Not detected, with detection limit given in parentheses
. Data from Reference 8

FIGURE 1

GENERAL STRUCTURES OF DIBENZO-*p*-DIOXIN (TOP) AND DIBENZOFURAN (BOTTOM)

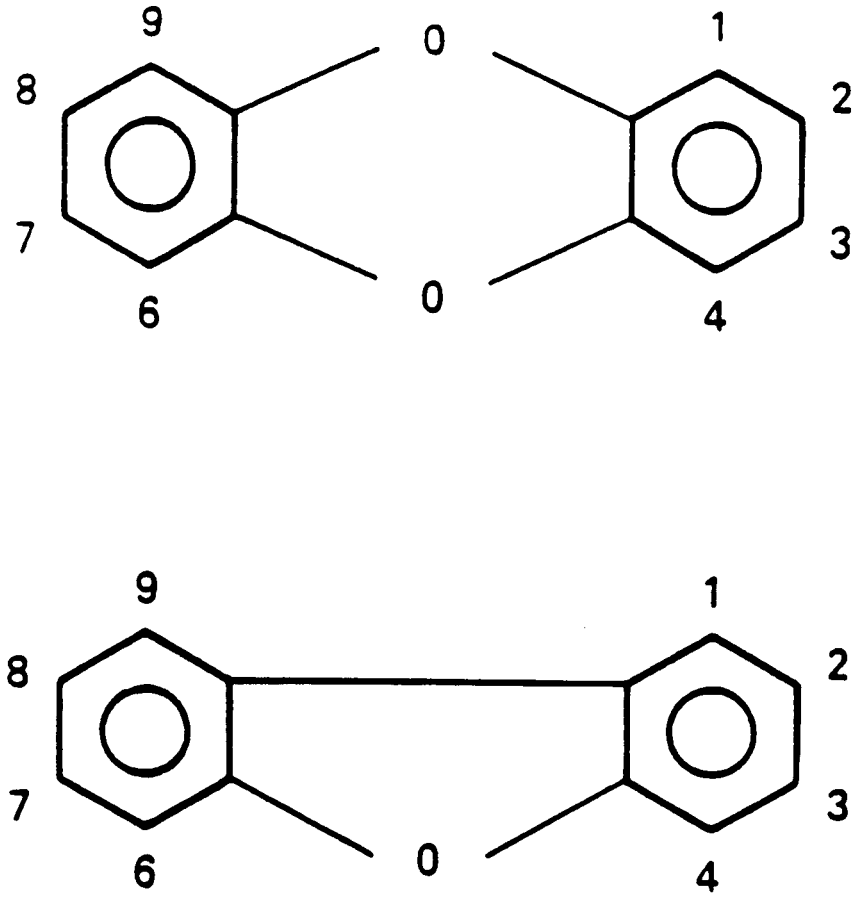
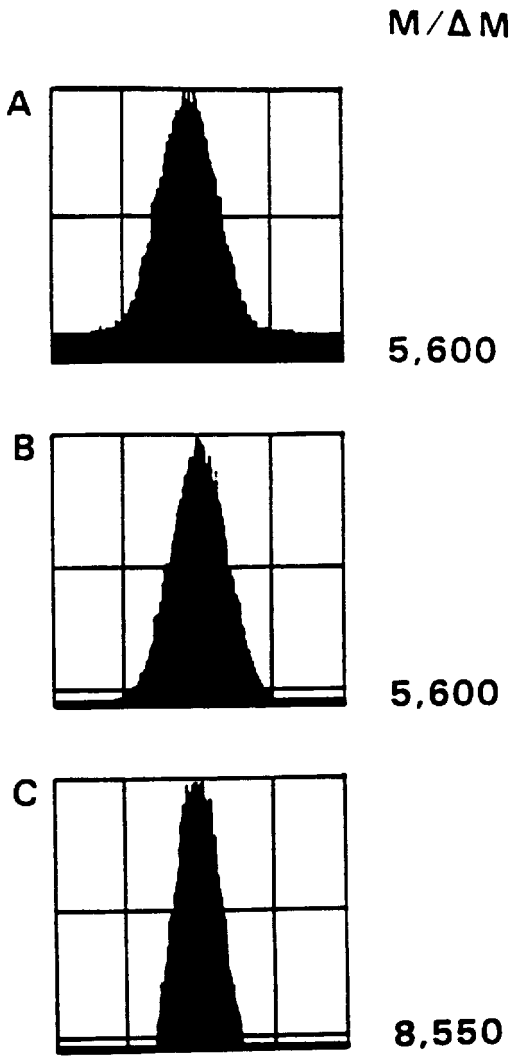


FIGURE 2




Peak profile displays demonstrating the effect of the detector zero on the measured resolving power. In this example, the true resolving power is 5,600.

A) The zero was set too high; no effect is observed upon the measurement of the resolving power.

B) The zero was adjusted properly.

C) The zero was set too low; this results in overestimating the actual resolving power because the peak-to-peak noise cannot be measured accurately.

FIGURE 3

TYPICAL 12-HOUR ANALYSIS SEQUENCE OF EVENTS.

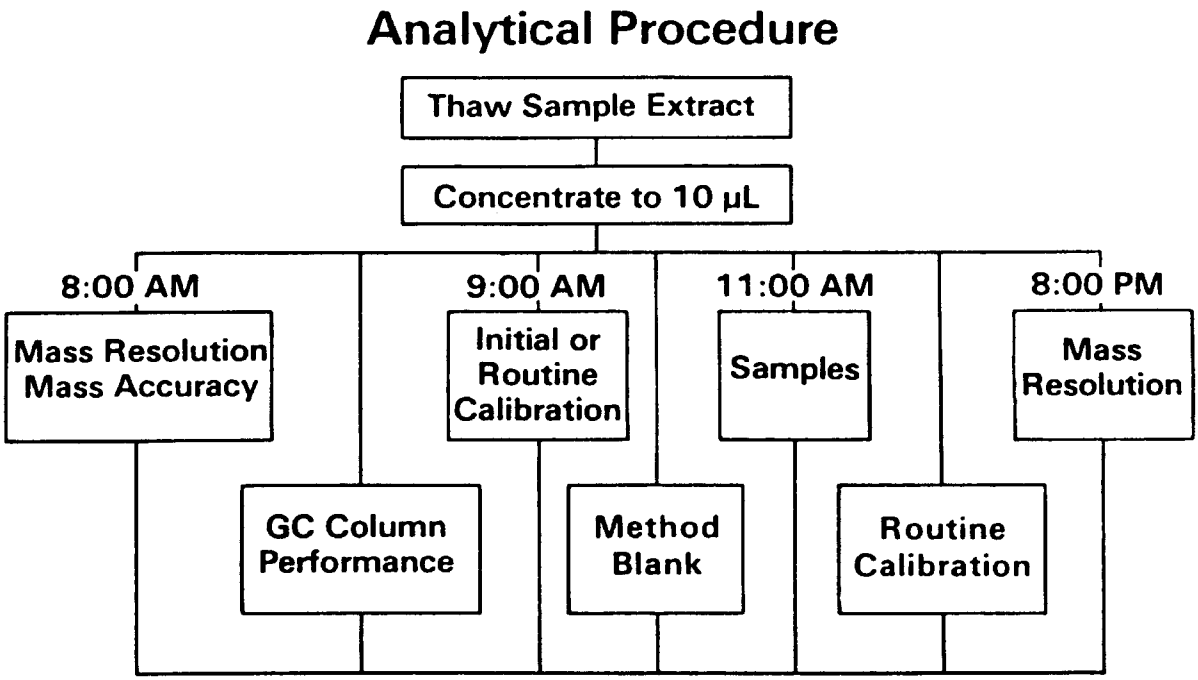
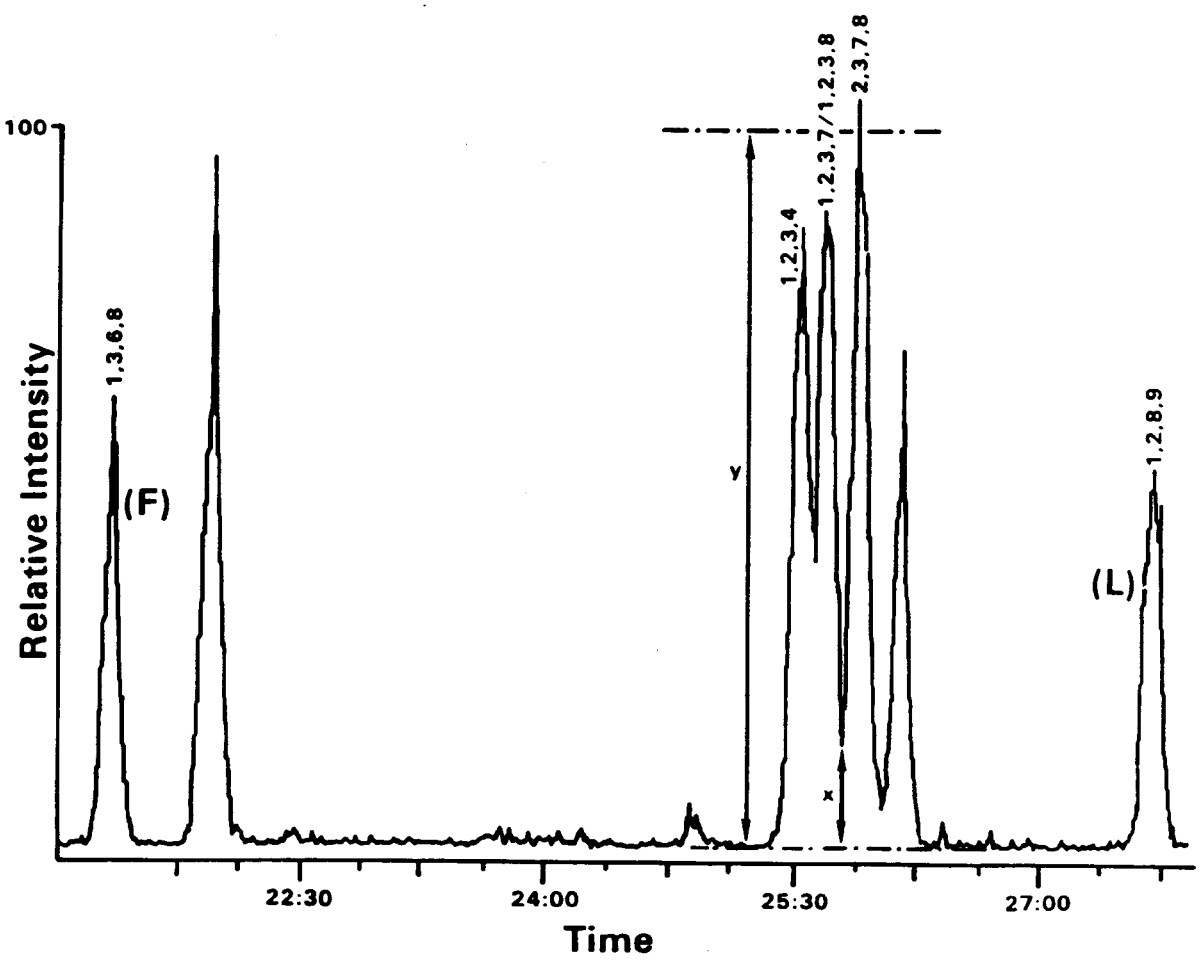
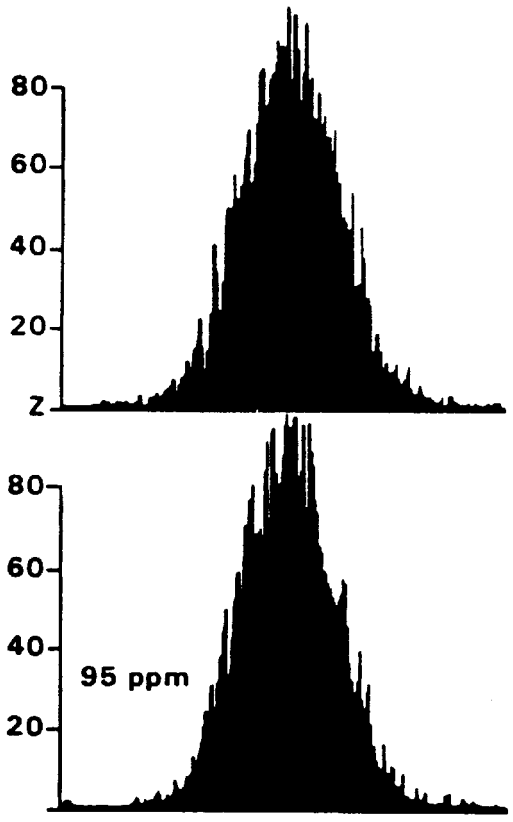


FIGURE 4




Selected ion current profile for m/z 322 (TCDDs) produced by MS analysis of the GC performance check solution on a 60 m DB-5 fused-silica capillary column under the conditions listed in Sec. 7.6.

FIGURE 5




**Ref. mass 304.9824 Peak top**
**Span. 200 ppm**

| | |
|---|---|
| System file name | YVES150 |
| Data file name | A:85Z567 |
| Resolution | 10000 |
| Group number | 1 |
| Ionization mode | EI+ |
| Switching | VOLTAGE |
| Ref. masses | 304.9824 |
| | 380.9260 |

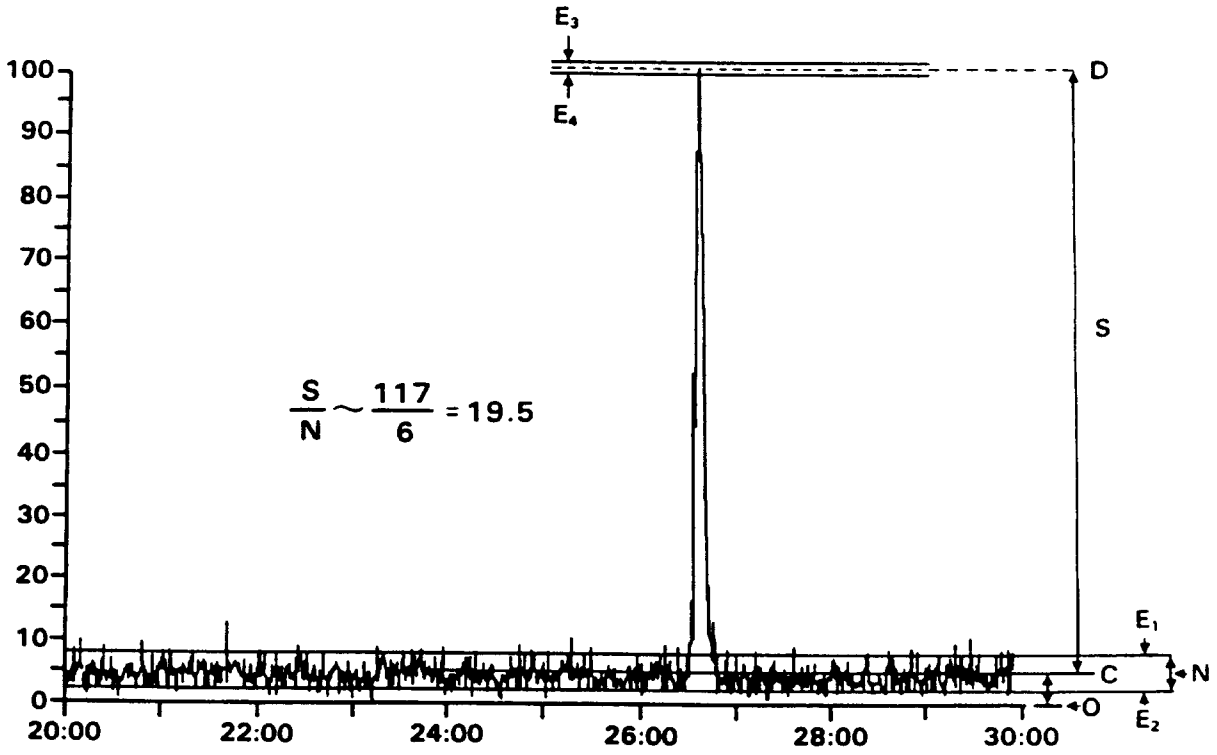
**M/ΔM~10,500**

**Channel B 380.9260 Lock mass**
**Span 200 ppm**

Peak profiles representing two PFK reference ions at m/z 305 and 381. The resolution of the high-mass signal is 95 ppm at 5 percent of the peak height; this corresponds to a resolving power M/⌂M of 10,500 (10 percent valley definition).

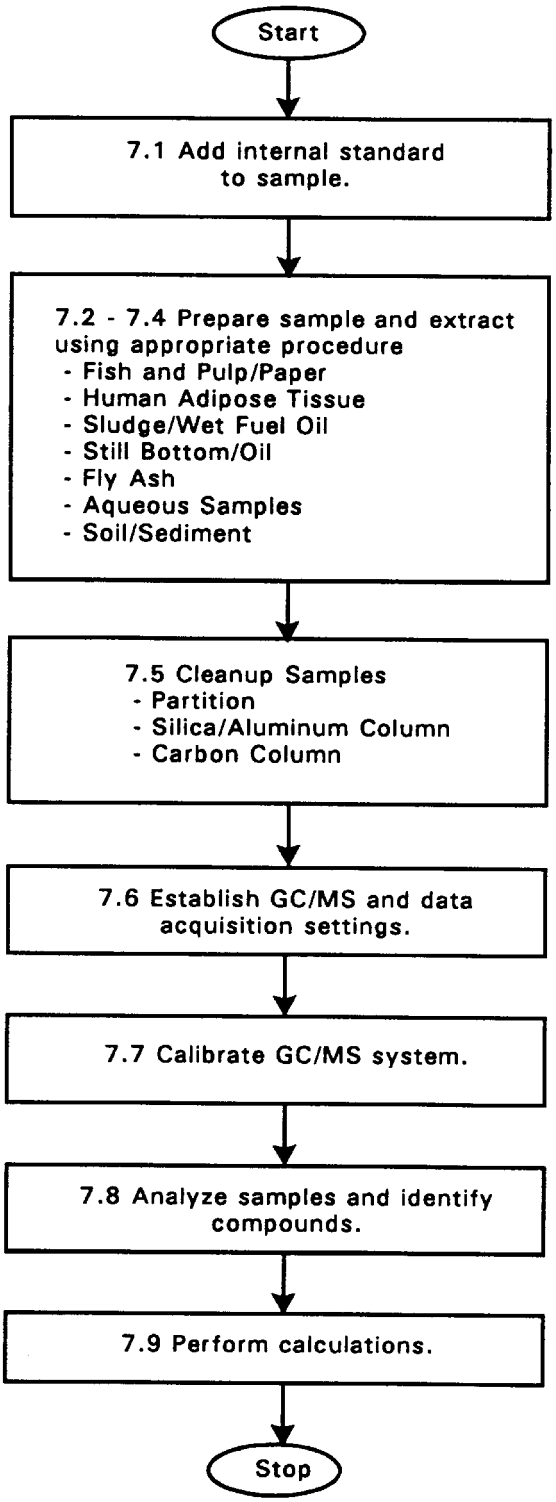
FIGURE 6

MANUAL DETERMINATION OF S/N.




The peak height (S) is measured between the mean noise (lines C and D). These mean signal values are obtained by tracing the line between the baseline average noise extremes, E1 and E2, and between the apex average noise extremes, E3 and E4, at the apex of the signal.

NOTE: It is imperative that the instrument interface amplifier electronic zero offset be set high enough so that negative going baseline noise is recorded.


Start
7.1 Add internal standard to sample.
7.2 - 7.4 Prepare sample and extract using appropriate procedure
- Fish and Pulp/Paper
- Human Adipose Tissue
- Sludge/Wet Fuel Oil
- Still Bottom/Oil
- Fly Ash
- Aqueous Samples
- Soil/Sediment
7.5 Cleanup Samples
- Partition
- Silica/Aluminum Column
- Carbon Column
7.6 Establish GC/MS and data acquisition settings.
7.7 Calibrate GC/MS system.
7.8 Analyze samples and identify compounds.
7.9 Perform calculations.
Stop